The Role of Horizontal Gene Transfer in Microbial Social Evolution

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Abstract

Plasmids are among the key vectors of bacterial horizontal gene transfer (HGT), a process often associated with evolutionary and ecological innovation as it provides a source of new genes and functions to the recipient of the transferred genetic material. Plasmids carry a wide range of genes, many of which are involved in bacterial social behaviour including medically relevant traits such as antibiotic resistance and virulence. This thesis aims to examine the evolutionary reasons why such traits are frequently mobile.

Cooperative public goods production generates a benefit which is shared with neighbours at an individual cost to the producer cell. Behaviours of this kind are frequently exploited by non-producer individuals as well as frequently transferred horizontally. Using mathematical models I investigate the role of HGT in the evolution and maintenance of bacterial cooperation. By partitioning the effects of HGT into those of infectivity and kin selection, and by incorporating analysis of bioinformatic data it emerges that there are three possible advantages for social traits relating to HGT. Firstly, infection with a plasmid obliges a cell to carry its particular genes, thereby allowing costly genes to spread. Secondly, the spread of plasmids can increase local relatedness by increasing the number of carriers of social genes within an interacting population, thereby increasing the frequency with which carriers of the cooperative gene interact with each other. As increased expression of a social gene allows higher levels of public goods, potentially increasing cooperation and productivity, a third advantage to plasmid carriage comes from the fact that plasmids are carried in multiple copies, thus allowing increased expression of the genes they carry, which can compensate for lower expression levels experienced by newly acquired genes.

I then investigate the horizontal spread of a different type of social trait: an anti-competitor bacteriocin. Bacteriocin producers will not spread if they are rare as they cannot sufficiently modify their environment to make up for the metabolic cost of bacteriocin production. I find that plasmids are an invaluable tool for bacteriocin dissemination as horizontal spread enables the bacteriocin producers to overcome a rarity threshold and become established in the population with plasmid addiction ensuring that the plasmid is maintained in the population.

Conflict can arise if cooperative genes are transmitted independently of the rest of the genome, leading to scenarios where horizontally spread cooperative genes are favoured where a chromosomal equivalent would not be. I find that repressing the expression of costly genes within a cell may be preferable to preventing the acquisition of the mobile element to resolve such genomic conflict. I then examine the role of plasmids in the evolution and maintenance of other useful genes: these are genes encoding antibiotic resistance, one of the key challenges facing medicine today. I use metapopulation models to explore the advantages of plasmid-based resistance over chromosome-based resistance and the means by which plasmid competition may hinder resistance spread.

The results of this thesis offer new perspectives on bacterial social evolution and the role of horizontal gene transfer therein. Plasmids may assist in the evolution and maintenance of social traits but such traits may also assist in plasmid maintenance, taking their relationship beyond the level of vector and gene.

Zusammenfassung

Plasmide gehören zu den wichtigsten Vektoren des horizontalen Gentransfers, einem Prozess, der oft mit evolutionärer und ökologischer Innovation in Verbindung gebracht wird, weil er eine Quelle neuer Gene und Funktionen für den Empfänger des genetischen Materials darstellt. Plasmide enthalten ein grosses Spektrum verschiedenartiger Gene, von denen viele am sozialen Verhalten von Bakterien beteiligt sind, unter anderem auch an medizinisch relevanten Eigenschaften wie Antibiotika-Resistenz und Virulenz. Diese Dissertation untersucht die evolutionären Gründe dafür, dass solche sozialen Gene oft über horizontalen Gentransfer übertragen werden.

Die kooperative Produktion öffentlicher Güter durch Bakterien bringt zwar einen Nutzen für die bakterielle Gemeinschaft, führt aber zu Kosten für die produzierenden Zellen. Es kann beobachtet werden, dass solch kooperatives Verhalten häufig von selbst nicht produzierenden Zellen ausgenützt wird, und dass es durch horizontalen Gentransfer verbreitet wird. Mit Hilfe mathematischer Modelle wird hier die Rolle des horizontalen Gentransfers in der Evolution und Verbreitung kooperativen Verhaltens unter Bakterien untersucht. Indem die Ansteckungsfähigkeit und die Verwandtenselektion als Auswirkungen des horizontalen Gentransfers separat untersucht werden, und unter Verwendung von Methoden der Bioinformatik, lässt sich zeigen, dass der horizontale Gentransfer drei mögliche Vorteile für soziale Verhaltensweisen bietet. Erstens zwingt eine Infektion durch ein Plasmid eine Zelle, die Gene des Plasmids weiter zu verbreiten, auch wenn diese Gene potentiell nachteilige Effekte für die infizierte Zelle haben. Zweitens kann die Verbreitung von Plasmiden lokal den Grad der Verwandtschaft von Zellen erhöhen, indem die Anzahl von Trägern sozialer Gene innerhalb einer interagierenden Population erhöht wird. Soziale Gene auf Plasmiden können das soziale Umfeld einer die Plasmide enthaltenden Zelle so beeinflussen, dass die Interaktion mit andern solchen Zellen erleichtert wird. Weil sich drittens Plasmide innerhalb einer Zelle selbst vervielfältigen können, sind sie in der Lage, sozialen Genen zu einer höheren Expression zu verhelfen, so die Produktion öffentlicher Güter zu vermehren und dadurch die Produktivität von Zellen zu verbessern. Auf diese Weise können Plasmide die allenfalls niedrige Expression eines neu erworbenen, sozialen Gens kompensieren.

Ich untersuche anschliessend die über horizontalen Gentransfer erfolgende Verbreitung von gegen konkurrierende Zellen gerichteten Bakteriocinen. Zellen, welche Bacteriocine erzeugen, können sich von alleine in einer Zellpopulation kaum verbreiten, so lange ihre Anzahl klein ist, da sie zusätzliche metabolische Kosten zur Produktion der Bakteriocine tragen müssen. Ich zeige, dass Plasmide eine wichtige Rolle beim Verbreiten der Gene zur Produktion von Bacteriocinen spielen, weil Plasmide diese Gene über den horizontalen Gentransfer verbreiten und diesen so erlauben, sich in einer Population zu etablieren.

Wenn soziale Gene anstatt durch vertikale Vererbung über Chromosomen durch horizontalen Gentransfer verbreitet werden, kann ein Konflikt entstehen zwischen diesen Genen und den übrigen Genen auf dem Chromosom. Ich zeige, dass es für eine Zelle vorteilhafter ist, diesen Konflikt zu entschärfen, indem sie die Expression von mit hohen metabolischen Kosten verbundenen, sozialen Genen in einer Zelle unterdrückt, als wenn sie die Aufnahme solcher sozialen Gene über horizontalen Gentransfer zu verhindern versucht. Anschliessend untersuche ich die Rolle von Plasmiden in der Evolution und in der Erhaltung von weiteren, potentiell nützlichen Genen für Bakterien, nämlich von Resistenzgenen gegen Antibiotika, zur Zeit eines der grössten medizinischen Probleme. Ich verwende Metapopulations-Modelle, um

die Vorteile einer Verbreitung solcher Resistenzen durch Plasmide gegenüber einer Verbreitung durch Chromosomen zu untersuchen. Zudem untersuche ich, wie die Konkurrenz zwischen Plasmiden die Verbreitung von Resistenzgenen gegen Antibiotika behindert.

Die Resultate dieser Dissertation zeigen neue Perspektiven auf bezüglich der sozialen Evolution von Bakterien und bezüglich der Rolle des horizontalen Gentransfers in dieser Evolution. Plasmide sind nützlich für die Evolution und Erhaltung von sozialen Genen, aber umgekehrt sind solche Gene auch nützlich in der Erhaltung von Plasmiden, so dass eine Wechselbeziehung entsteht zwischen Plasmiden und sich darauf befindlichen, sozialen Genen, welche weit über das blosse Verhältnis von Übertragungsvektor und übertragenem Gen hinausgeht.

Mura gcuirfidh tú san earrach ní bhainfidh tú san fhómhar.

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Author Contributions

	Chapter 2	Chapter 3	Chapter 4	Chapter 5	Chapter 6
Original Idea	SMG, DR	SMG, DR	SMG, DR	SMG, DR	SMG, DR
Study Design	SMG, LL, DR	SMG, DR	SMG, DR	SMG, DR	SMG, DR
Data Collection	-	EF, ER	-	-	-
Analysis	SMG, LL, DR	SMG, DR	SMG, DR	SMG, DR	SMG
Manuscript preparation	SMG, LL, SB, DR	SMG, DR, ER, AW	SMG, DR, RFI	SMG, DR	SMG, DR

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1. Introduction

1.1 Preface

This thesis will focus on two facets of bacterial life, operating at different levels. Both involve interaction with other individuals, at the gene level and the gene product level. The first topic is that of horizontal gene transfer, a ubiquitous feature of bacteria. The second is that of social behaviour, potentially more limited in scope but no less fascinating. Horizontal gene transfer may play an important role in the evolution of bacterial social behaviour (Nogueira et al. 2009). The following pages will illustrate the importance of both these aspects of the bacterial lifestyle and discuss the importance of their interaction.

1.2 Horizontal gene transfer

Horizontal gene transfer (HGT) refers to the non-genealogical transfer of genetic material from one organism to another (Goldenfeld and Woese 2007). HGT is a promiscuous process, it has been shown to occur among and between domains in all possible directions, even between Bacteria and Eukarya (Boto 2010) but it is mostly widely studied among bacteria and archaea. HGT is often associated with evolutionary and ecological innovation as it provides a source of new genes and functions to the recipient of the transferred genetic material (Boto 2010). For example, HGT can enable cells to survive and thrive in the presence of antibiotics, heavy metals or new food sources (Eberhard 1990). Prokaryotic genomes display varying degrees of gene loss and gain but it is estimated that up to 32% of any microbial genomes has been acquired horizontally (Koonin et al. 2001). In particular, a study in *Escherichia coli* has demonstrated that non-core genes (those with a high propensity to be mobile) may make up to 90% of the pan-genome available to that species (Touchon et al. 2009). Genes can be

transferred horizontally in three main ways: via the uptake of naked DNA from the environment (transformation), or through the spread of certain vectors: viruses (transduction) and transfer of plasmids (conjugation). Only transformation is under the control of the bacteria itself (Sota and Top 2008).

1.2.1 Plasmid-mediated horizontal gene transfer

Throughout, we focus on plasmids as a means of HGT. Plasmids are a form of nonessential extrachromosomal DNA which replicate autonomously within a bacterial cell and spread by means of conjugation (Novick 1987; Sota and Top 2008). Naturally occurring plasmids can range from one to several hundred kilobases in size and in copy number from one to several hundred per cell (Novick 1987).

Plasmids can be inherited vertically during cell division and also transmitted horizontally between cells. Plasmids which contain both mobility (MOB) genes, which allow conjugative DNA processing, and mating pair formation (MPF) genes (for the formation of a type IV secretion system to form a mating channel between donor and recipient cells) are termed conjugative. Plasmids which contain MOB genes, but use the mating channel of another plasmid to move horizontally, are termed mobilizable. Those plasmids which are neither conjugative nor mobilizable are termed non-mobilizable and spread by transduction or transformation. It has been estimated that a quarter of plasmids are conjugative, a quarter are mobilizable and half of all plasmids are non-mobilizable (Smillie et al. 2010).

Plasmids are present in all branches of the bacterial 'tree of life' and have been found in all bacterial communities studied to date (Sorensen et al. 2005). The ability to spread over a diverse host range eliminates the danger of becoming extinct should environmental conditions in one population change unfavourably (Norman et al. 2009). The rates of donation between and within bacterial strains can vary greatly within plasmids (Dionisio et al. 2002). In addition, vertical spread is reduced by the occasional loss of plasmids due to segregation (Simonsen 1991). The horizontal component of plasmid transmission is dependent on the density of potential recipients and the rate at which transfer occurs. As such, hotspots of HGT tend to be found in areas of high bacterial density, such as biofilms (Sorensen et al. 2005) or during microbial blooms (Stecher et al. 2012).

Plasmids impose fitness costs on their bacterial hosts related to carriage and the time and resources required to replicate extra DNA (Lili et al. 2007). There are also potential costs associated with transfer of plasmids, such as the production of conjugative pili and the risk of viral infection via such pili (Dahlberg and Chao 2003; Wagner and Hewlett 2004). Indeed DNA that is horizontally transferred can be extremely costly as it may, under certain conditions, drive populations to extinction (Rankin et al. 2010) and it has been shown that there is a correlation between transmission rates and cost to the host (Turner et al. 1998). As they are incapable of an independent existence outside of the bacterial cell, the evolutionary fates of plasmids are inseparably associated with those of their bacterial hosts (Modi and Adams 1991). Plasmids replicate within their host cells but a trade-off exists between the number of copies of the plasmid and costs to the host cell (Paulsson 2002) as the plasmid is not served by imposing a cost so high as to kill its host. In fact, when multiple plasmid types infect a cell through high infectivity, within-host plasmid competition can reduce host fitness dramatically, creating a tragedy of the commons with plasmids becoming victims of their own

success (Smith 2012). But plasmids do reproduce and transmit themselves by using host resources so that there is inevitably a limit on reducing virulence (Frank 1996). In this sense they are molecular parasites, thus one may expect changes to the bacterial and plasmid chromosome to facilitate a reduction in the deleterious effect of the plasmid (Bouma and Lenski 1988; Modi and Adams 1991). Indeed coevolution tends to lead to an amelioration in plasmid costs over time (Dahlberg and Chao 2003; Dionisio 2005).

1.2.2 Plasmid maintenance

Despite the near-ubiquity of plasmids in bacterial populations and the profound contribution of HGT to the adaptation and evolution of bacteria (Ochman et al. 2000), the mechanisms responsible for the maintenance of plasmids in bacterial populations are poorly understood (Bergstrom et al. 2000; Sorensen et al. 2005). They may be maintained by various selection regimes (Bergstrom et al. 2000) or through transmission alone (Levin 1993; Bahl et al. 2007), although this is debated (Lili et al. 2007). Alternatively they may be maintained because they somehow benefit their host (Simonsen 1991), although it is possible that useful genes will be incorporated into the bacterial chromosome (Bergstrom et al. 2000). However, the absence of such chromosomal integration from studies under positive selection (e.g. Bouma and Lenski 1988; Turner et al. 1998; Dionisio et al. 2005) suggests this may in fact be relatively rare.

Plasmids possess a variety of mechanisms to ensure that they are passed on to the next generation during cell division. For instance low copy number plasmids use an active partition process to ensure that plasmid copies are distributed to daughter cells (Austin and Nordstrom 1990; Bouet et al. 2007). Plasmids may be maintained through a process known as post-segregational killing (Gerdes et al. 1986), also known as plasmid addition systems. This

requires at least two plasmid genes: one specifying a toxin and the other an antidote to this toxin. The toxin is stable and remains in daughter cells after cell division but the antidote (either a protein or antisense RNA) is unstable and thus without the presence of the plasmid (and the antidote it carries), the cell is killed (Zielenkiewicz and Ceglowski 2001).

Plasmids may also be lost due to plasmid incompatibility: the failure of two coexistent plasmids to be stably inherited in the absence of external selection (Novick 1987). Incompatibility between plasmids arises when they share either the same mechanism of replication control or the same partition mechanism (Novick 1987; Schumann 2001). However, some measure of variability may exist in the degree to which plasmids containing the same origin of replication are unstable in the host because different plasmids containing identical origins of replication have been shown to be stably maintained in bacteria for periods that are experimentally significant (Velappan et al. 2007). Studies have also shown that plasmids can evolve an increased ability to "superinfect" bacteria already infected with incompatible plasmids (Smith 2011).

Because of their potentially parasitic nature it is to be expected that hosts would evolve some form of resistance or immunity to plasmid transfer. Clustered regularly interspaced short palindromic repeats (CRISPRs) are arrays of prokaryotic DNA sequences of highly conserved 24- to 47-bp repeats, separated by variable, often unique spacer sequences, derived from foreign replicons such as phage or plasmids (Vale and Little 2010). In combination with CRISPR associated (Cas) proteins CRISPRS mediate a form of acquired immunity to specific viral pathogens (Sorek et al. 2008; van der Oost et al. 2009) and also to plasmids by limiting conjugation (Marraffini and Sontheimer 2008; Garneau et al. 2010). This specific defence is something more usually associated with vertebrate immune systems (Vale and Little 2010).

The prevention of conjugation and phage infection by CRISPRs suggests a capacity for these loci to reduce the acquisition of genetic traits that allow bacteria to become virulent (Marraffini and Sontheimer 2008).

1.2.3 Commonly exchanged traits

Plasmids genomes are made up of a backbone of essential genes which control core plasmid functions but they also carry a wide range of different 'accessory' genes, and many factors are likely to influence which genes are carried on plasmids and why (Turner et al. 2002; Rankin et al. 2011b). Accessory genes are commonly involved in interactions between the bacteria and other organisms for example, new metabolic functions such as nitrogen fixation in Rhizobia (Long 1989; MercadoBlanco and Toro 1996) or manipulation of other organisms (such as manipulation of plant cells by *Agrobacterium* species (Gelvin 2003)). Alternatively these accessory genes may be active in interactions between the bacteria and its environment such as resistance to environmental toxins for example antibiotics (Barlow 2009; Svara and Rankin 2011) or heavy metals (Tett et al. 2007). One of the most important roles of plasmid accessory genes is to confer virulence traits (Elwell and Shipley 1980; Buchrieser et al. 2000; Barth and Bauerfeind 2005; Silby et al. 2011), by which they enable bacteria to colonize and grow in the host. Virulence traits are often an important component of microbial social behaviour (e.g. see West et al. 2007a).

1.3 Social evolution

From an evolutionary point of view, a behaviour is social if it has fitness consequences for both the individual that performs that behaviour (the actor) and another individual (the recipient(s)) (West et al. 2007b). The different effects that social behaviours have on individuals are illustrated in Figure 1. Social evolution examines both cooperation and conflict at different scales (West et al. 2007b). Throughout we will frequently refer to one of the most common forms of bacterial social behaviour, that is, cooperation. Cooperation is a social behaviour which provides a benefit to another individual (recipient), and which is selected for because of its beneficial effect on the recipient (West et al. 2007b). Cooperation is common in all organisms at multiple levels of organisation (Sachs et al. 2004; Lehmann and Keller 2006; West et al. 2007a). It is well established that cooperation can only occur in one or other of two scenarios: if there are direct fitness benefits to the cooperating individual (i.e. mutual benefits for actor and recipient) or else indirect fitness benefits to the actor (Gardner and Foster 2008), by improving the fitness of other individuals who carry the gene coding for the cooperative trait (Hamilton 1964; Abbot et al. 2011). This is often termed kin selection (Maynard-Smith 1964). This idea is captured by Hamilton's rule, $br > c$, where b describes the benefit stemming from the cooperative action, r, the relatedness between individuals (with respect to the cooperative gene), and c, the cost (Hamilton 1964). This thesis examines social evolution at the microbial level.

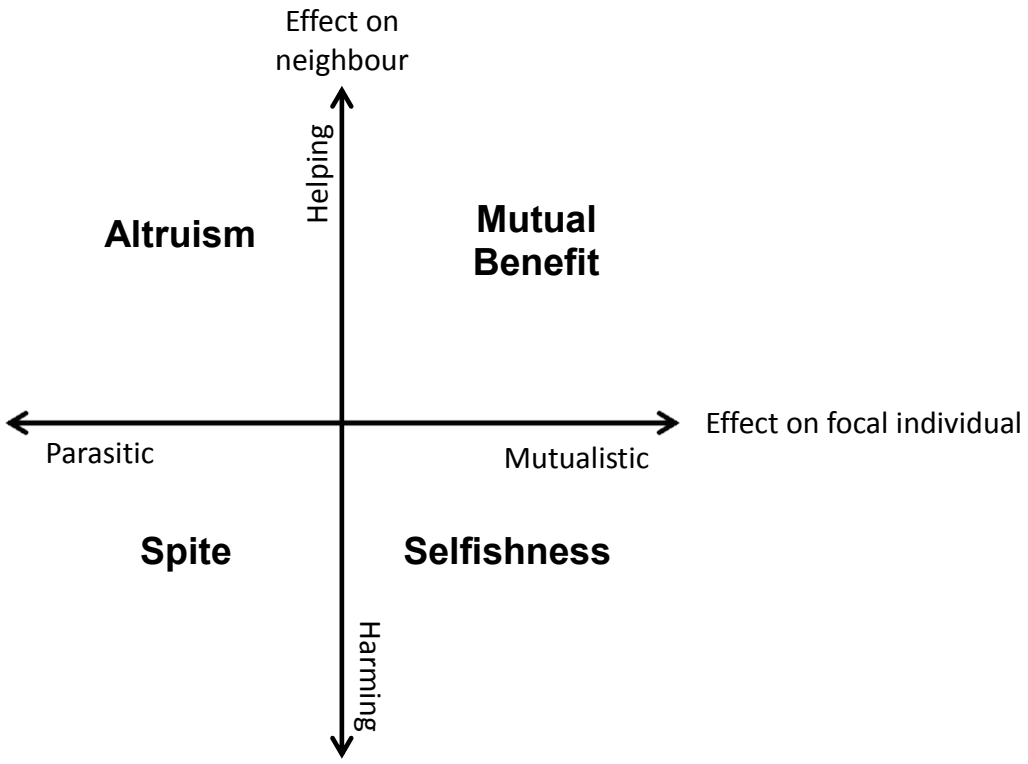

Figure 1. The social spectrum

General classification of social traits and their effect on the focal individual or a social neighbour. Figured based on Figure 2, Rankin, D. J., E. P. C. Rocha, and S. P. Brown. (2011) *Heredity* 106:1-10. Cooperative behaviours such as public goods production can be mutually beneficial (when the producer receives some direct benefit) or altruistic (when the producer receives no direct benefit). Non-producers which benefit from the public good without producing it are termed selfish. Anti-competitor traits such as colicin production (Cascales et al. 2007) can be termed spiteful as they damage competing individuals but only at the individual cost of cell lysis.

1.3.1 Microbial sociality

Social evolution theory has stemmed from a desire to explain the complex social behaviours exhibited by vertebrates. However, microorganisms are now known to display all of the hallmarks of a complex and coordinated social life (Crespi 2001; West et al. 2006; West et al. 2007a). Many examples of social evolutionary theory have been demonstrated using microorganisms as a model system (Rainey and Rainey 2003; Griffin et al. 2004; Diggle et al. 2007b), and it is well recognised that many traits in bacteria can be interpreted as having a social effect. Microbes have recently been demonstrated to exhibit green beard discrimination (Smukalla et al. 2008); anti-competitor behaviour e.g. bacteriocin production (Riley and Wertz 2002) or release of temperate phage (Brown et al. 2006); cooperative communication (Diggle et al. 2007a; Diggle et al. 2007b; Velicer and Vos 2009); and even cannibalism (González-Pastor 2011). Some bacteria even show phenotypic plasticity in their propensity to cooperate (Kummerli et al. 2009). In addition, a variety of ecological factors can influence the success of cooperative behaviours such as frequency dependency (Ellis et al. 2007), the availability of nutrients (Brockhurst et al. 2008); the durability of cooperative products (Kuemmerli and Brown 2010); environmental disturbance (Brockhurst et al. 2007); and population viscosity (Kuemmerli et al. 2009).

The production of public goods, the benefit of which is shared with other member of the population while the cost is borne solely by the individual producer, is possibly the most common form of social behaviour in microbes (West et al. 2007a). Production is costly but the result is a benefit to all individuals in the local population, leaving producers open to exploitation by non-producers in a mixed population of producer and mutant non-producer bacteria as the non-producers can gain the benefit of public good without paying the cost and

hence increase in frequency (West et al. 2007a). Such public goods can be seen in a wide range of bacterial products such as siderophore production (Griffin et al. 2004; Buckling et al. 2007), biofilm formation (Brockhurst et al. 2006; Xavier and Foster 2007) or antibiotic resistance (Ciofu et al. 2000).

1.4 Horizontal gene transfer as a means of spreading and maintaining cooperation

A recent study, which looked into the set of proteins expressed in 22 *Escherichia* and *Shigella* genomes, found that secreted proteins were over-represented on mobile elements (Nogueira et al. 2009). Secreted proteins, those which are localized in the extracellular environment, represent a proxy for social traits because they are proteins that are likely to interact with other cells in the population (public goods are an example of secreted proteins). Thus this study revealed that social traits are more likely than expected to be plasmid-based. An accompanying model found more mobile loci create higher relatedness among their neighbours at those loci by virtue of their mobility. Therefore, one can expect a cooperative trait to be maintained via kin selection when the trait is carried on a mobile locus. This result seems to confirm a previous theoretical study (Smith 2001), which suggested that horizontal transfer is an important mechanism for the maintenance of cooperation in microbes. Smith's study first captured the familiar social dynamics of chromosomally-determined cooperators and defectors (non-cooperators), illustrating that a population of individuals which produced a public good could easily be invaded by individuals which did not produce it, resulting in the breakdown of the public good (Smith 2001), an outcome known as 'the tragedy of the commons' (Hardin 1968; Rankin et al. 2007). Smith (2001) then demonstrated that allowing plasmids to carry the gene producing the public good could lead to the 'tragedy' being

averted, and cooperation maintained in the face of non-producers – as the non-producers would become infected with the plasmid and therefore with the cooperative gene. Further studies have demonstrated that indeed, cooperation can be maintained by plasmids in the way suggested by Smith, but that this mechanism can be disrupted in some scenarios, specifically when competition with an incompatible non-cooperative plasmid is introduced, it appears plasmid carriage makes no difference to the prevalence of a cooperative phenotype (Mc Ginty et al. 2011). In these circumstances the social dilemma is shifted to the plasmid level and the scenario reverts to competition between cooperators and defectors, where defectors triumph as expected.

There are three advantages of HGT as a means of transfer of social traits: infectivity, kin selection and gene dosage. These are discussed in the following sections.

1.4.1 Infectivity

We can refer to the infectious transfer of plasmids (and indeed other mobile genetic elements) as their infectivity. The infectivity of plasmids (combined with competence from their host cells to receive the plasmid and the absence of defence mechanisms or previously established incompatible plasmids) is the key to horizontal gene transfer. By virtue of their infectivity plasmids can force a cell to carry certain genes and, in this way, can spread even costly traits, provided the rate of plasmid transfer is sufficiently high. Social traits, such as those for the production of a public good, are often costly but may disseminate through a population through the process of plasmid infectivity. Figure 2 illustrates this process for a putative virulence factor. This is a powerful process as some plasmids persist as parasites even if they bring no benefit to their host (e.g. IncP-1 plasmids, Bahl et al. 2007; Fox et al. 2008). It has

been argued that this is the only advantage to horizontal transmission for a cooperative trait (Giraud and Shykoff 2011).

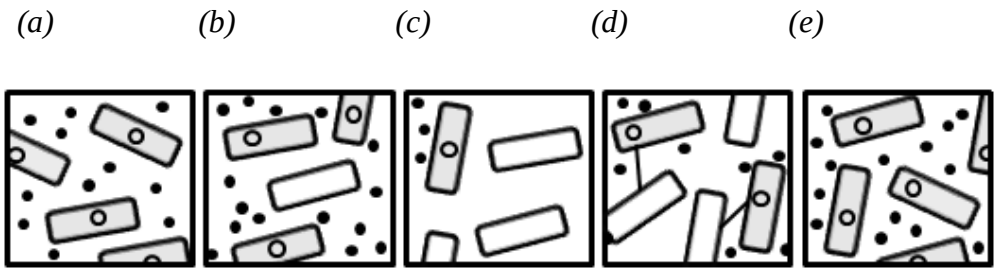

Figure 2. The maintenance of public goods by horizontal gene transfer

(*a*) Bacterial cells (grey) produce an extracellular virulence factor (black dots). (*b*) Mutant cheaters (white) that do not produce the virulence factor arise and increase in frequency (*c*) because they do not pay the cost of producing the virulence factor. (*d*) Horizontal gene transfer reintroduces functioning virulence-factor genes to cheaters and (*e*) converts them into producers of the virulence factor. Figure based on Figure 1, Smith, J. (2001) *Proceedings of the Royal Society of London Series B-Biological Sciences* 268:61-69.

1.4.2 Kin Selection

The success of horizontally transmitted social traits may be bolstered by a second force, that of kin selection (Rankin et al. 2011a). Kin selection is an evolutionary force that acts when individual fitness is affected by traits expressed by relatives (Taylor et al. 2007). Relatedness is measured at the level of the focal gene (that is, the social trait) and encompasses the extent to which random recipients (individuals whose fitness is affected by the effects of the social

trait) are more (or less) likely to carry the same plasmid as the focal cooperator than is an average cell in the patch (Pepper 2000). Kin selection is important in the success of many chromosomally-based social traits. Siderophore production is a cooperative trait necessary for virulence in acute *Pseudomonas aeruginosa* infections and low relatedness has been shown to lead to decreased virulence in such infections (Harrison et al 2006). In fact, many other traits associated with growth and virulence in pathogenic bacteria appear to be subject to kin selection including biofilms and immune suppression (Griffin et al 2004). The spread of plasmids (carrying the social trait) may increase local relatedness by increasing the numbers of carriers of social genes within an interacting population, as illustrated by Rankin et al. (2011a). Thus the spread of plasmids (via the aforementioned infectivity process), may increase relatedness, leading kin selection to support the cooperative trait. This implies that infectivity and kin selection effects are complementary processes in the establishment and maintenance of mobile cooperative traits.

1.4.3 Gene Dosage

Gene dosage describes the number of copies of particular genes carried within an individual cell (Kondrashov and Kondrashov 2006). Therefore the gene dosage of a plasmid-carried gene depends on the plasmid copy number. A single cell can carry multiple copies of a plasmid, in some cases up to 700 (del Solar et al. 1998; Friehs 2004), this in turn allows increased expression of plasmid-based genes (Nguyen et al. 2006; Hastings et al. 2009). As such a third potential advantage to plasmid-based spread derives from the potential boost in gene dosage resulting from plasmid copy number. As cooperators are known to display high productivity (Harrison et al. 2006), increased expression of cooperative traits could lead to increased productivity. As public goods are secreted outside of the cell, higher expression of

such traits, and thus more abundant public goods, may result in an increased direct benefit from the behaviour. In addition, horizontally transmitted genes are often poorly expressed (Thomas and Nielsen 2005; Lucchini et al. 2006); increased gene dosage can alleviate this problem.

1.5 Thesis Overview

The scope of this thesis encompasses a broad range of evolutionary topics. The primary aim was to theoretically examine the means by which horizontal gene transfer supports social traits to add to our overall understanding of why social straits are so prevalent on mobile genetic elements (Chapters 2-4). I also aspired to broaden understanding of both microbial social dynamics and plasmids themselves (Chapter 5-6). The former topic is addressed through the study of the conflict which may be engendered between a horizontally transmitted trait and its host chromosome and the means by which it is can be resolved. The latter topic is addressed by examining how the benefits for a non-social trait to be horizontally transmitted. This thesis therefore can add to the canon of knowledge regarding social evolution and plasmid dynamics and can aid in bridging the gap between two fascinating areas of microbial evolution. Individual chapters and their publication status are outlined in the below sections.

1.5.1 Chapter 2: The Interplay between Relatedness and Horizontal Gene Transfer Drives the Evolution of Plasmid-Carried Public Goods[1]

This chapter considers the effects of plasmid transfer of genes for public goods cooperation, focusing on the infectivity of the plasmid and the alteration in relatedness that stems from horizontal transfer. I use a modeling approach based on the Price Equation (Price 1970; Price 1972) which supplies an explicit means to model the evolution of plasmid-borne bacterial cooperation. The results demonstrate that, due to its effect on relatedness, plasmid mobility increases the invasion and stability of public goods, in a way not seen in individually beneficial traits. In addition, plasmid transfer increases relatedness when public goods production is rare but this effect declines when production is common, with both scenarios leading to an increase in the frequency of plasmid-borne public goods. The findings of this model highlight the importance of both transmission and relatedness as factors driving the evolution of plasmid-borne cooperation.

1.5.2 Chapter 3: The role of Horizontal Gene Transfer and Gene Dosage in the Evolution of the Secretome[2]

In this chapter I investigate the advantage of plasmid transmission in terms of potentially increasing both the gene dosage and the expression levels of a cooperative trait. Firstly, I build a simple population genetics-based model to compare chromosomal based gene duplications or plasmid based HGT as a mechanism for increasing gene dosage of cooperative traits. Subsequently, using secreted proteins as a proxy for sociality (Nogueira et al. 2009), I

[1] Submitted to *Proceedings of the Royal Society B*
[2] To be submitted

analyse a dataset of >1 million proteins from 291 bacterial strains. These two approaches allow us to examine in depth how bacterial social behaviour may be promoted through HGT and gene dosage effects. We find that secreted proteins tend to plasmid-borne, more recently acquired and less costly in addition to being more highly expressed. Smaller plasmids, which can have higher copy numbers within a cell, also tend to carry proportionately more secreted proteins. These results suggest that horizontal transmission combined with high product expression through gene dosage and potentially increased gene expression levels has supported the spread of secreted proteins and thus promotes social behaviour among bacteria.

1.5.3 Chapter 4: Horizontal Gene Transfer Promotes the Evolution and Spread of Bacteriocins[3]

Bacterial social behaviour may also take the form of a negative interaction with a competitor. For this purpose bacteria have amassed a diverse array of weapons, including antibacterial toxins, known as bacteriocins (Riley and Wertz 2002; Riley et al. 2003). The spread of bacteriocin producers is constrained by the cost to an individual of successfully modifying the environment. Bacteriocin producers will only spread once there are enough of them in a population to sufficiently damage competitors (and thus compensate for the cost of bacteriocin-production). This phenomenon is known as a rarity threshold (Brown et al. 2009). Here I model the spread of plasmid-carried bacteriocins. I find that horizontal transfer of the bacteriocin trait via plasmids enables it to successfully overcome the rarity threshold and become established in the population. At this point horizontal transfer is no longer required for the trait to remain stable as the bacteria are addicted to the plasmid. This means that if any cell loses the plasmid they will be killed by the toxin because the plasmid also encodes an

[3] Submitted to *BMC Evolutionary Biology*

anti-toxin which neutralizes the bacteriocin (Gerdes et al. 1986; Gerdes et al. 2005). Thus horizontal gene transfer allows bacteriocins to spread in a population and bacteriocins can allow plasmids to persist even after horizontal transfer is not necessary for the maintenance of the bacteriocin itself.

1.5.4 Chapter 5: The Evolution of Conflict Resolution between Plasmids and Their Bacterial Hosts[4]

Conflict between genes is an important factor in shaping genomes. In the case of cooperative genes, a conflict may arise if they are transmitted independently of the rest of the genome, leading to the whole genome being exploited by surrounding non-cooperative individuals. A variety of mechanisms exist to defend against mobile elements (Johnson 2007) that have the potential to resolve this conflict. In this chapter I use modeling techniques based on the Price Equation (Price 1970; Price 1972) to focus on two such mechanisms: one targeting the plasmid itself and the other the cooperative gene. The first mechanism explored is direct resistance to plasmid infection, that is, carriage of a trait that prevents conjugation or breaks down foreign DNA. I also explore a scenario where the conflict is resolved through interaction between the host genes and the plasmid genes leading to suppression of the plasmid-carried cooperative gene by a chromosomal allele. I find that gene suppression, which allows the spread of the plasmid whilst mollifying its cooperative trait, is the most stable mechanism of conflict resolution whereas resistance leads to cycling between resistant and non-resistant cells.

[4]Mc Ginty, S. É., and D. J. Rankin (2012) The Evolution of Conflict Resolution between Plasmids and Their Bacterial Hosts. *Evolution.* 66, 1662-1670

1.5.5 Chapter 6: Horizontal Gene Transfer and the Evolution of Antibiotic Resistance[5]

Antibiotic resistance is one of the traits most commonly spread by horizontal transmission (Eberhard 1990; Bennett 2008). In fact, multiple antibiotic resistance genes are frequently arranged in clusters on plasmids (Barlow 2009), thus allowing the rapid dissemination of a variety of resistance traits together. In this chapter I investigate the spread of antibiotic resistance plasmids in a metapopulation using a model based upon their within-host dynamics. I investigate competition between resistance plasmids and resistance genes encoded on the chromosome in addition to incorporating the effects of plasmid competition. I find that resistance plasmids will outcompete chromosomal resistance but that reduced interaction between chromosomal and plasmid based resistance can allow chromosomal resistance to spread. Competition with an incompatible plasmid also decreases the frequency of plasmid-based resistance and suppresses resistance in the absence of antibiotics while boosting chromosomal resistance in the presence of antibiotics. Overall the results suggest that plasmids are the main drivers of resistance in a metapopulation.

[5] Submitted to *PLoS ONE*

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2 The Interplay between Relatedness and Horizontal Gene Transfer Drives the Evolution of Plasmid-Carried Public Goods

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Submitted to *Proceedings of the Royal Society Series B*

2.1 Abstract

Plasmids carry a wide range of genes which are often involved in bacterial social behaviour. The question of why such genes are frequently mobile has received increasing attention. Here we use an explicit population genetic approach to model the evolution of plasmid-borne bacterial public goods production. Our findings highlight the importance of both transmission and relatedness as factors driving the evolution of plasmid-borne public goods production. We partition the effects of plasmid transfer of social traits into those of infectivity and the effect of increased relatedness. Our results demonstrate that, due to its effect on relatedness, plasmid mobility increases the invasion and stability of public goods, in a way not seen in individually beneficial traits. In addition we show that plasmid transfer increases relatedness when public goods production is rare but this effect declines when production is common, with both scenarios leading to an increase in the frequency of plasmid-borne public goods. Plasmids remain important vectors for the spread of social genes involved in bacterial virulence thus an understanding of their dynamics is highly relevant from a public health perspective.

2.2 Introduction

Mobile genetic elements, such as plasmids, are ubiquitous in bacterial genomes (Frost et al. 2005; Molbak et al. 2003; Slater et al. 2008; Sorensen et al. 2005). Plasmids carry a wide range of different 'accessory' genes, and many factors are likely to influence which genes are carried on plasmids and why (Rankin et al. 2011b; Turner et al. 2002). Plasmids are disproportionately likely to carry genes that code for secreted proteins (Nogueira et al. 2009). As secreted proteins are costly to produce and provide a benefit to other individuals, they raise the question of why an individual should carry out a behaviour that is potentially costly to perform but benefits others. Theoretical explanations for public goods production (PG) have shown that such behaviour can evolve if there are either direct fitness benefits to the producer individual (i.e. mutual benefits for actor and recipient) or else indirect fitness benefits to the actor, so that kin selection is operating (Frank 1998; Grafen 1985; Hamilton 1964). These social traits are of particular relevance in a public health sense as secreted factors are often known to be virulence determinants (Nogueira et al. 2009; Smith 2001). Plasmids are also known for their carriage of antibiotic resistance traits, and these traits can also, in some cases, be characterized as public goods due to the benefit they may confer on neighbouring cells as, for example, β-lactamase exported from *Pseudomonas aeruginosa* in outer membrane vesicles (Ciofu et al. 2000).

Previous work has demonstrated that genes involved in bacterial PG and virulence are over-represented on mobile elements, or areas of the bacterial genome likely to have originated by horizontal transfer (Ho Sui et al. 2009; Nogueira et al. 2009). This

association highlights the importance of gene mobility in bacterial social evolution (Nogueira et al. 2009; Smith 2001). It has been argued that horizontal gene transfer (HGT) of public-goods producing genes could act as a novel mechanism for the evolution of PG (Smith 2001). HGT of public-good producing plasmids has the effect of converting previously non-producing cells into producers of a public good (Smith 2001). As such, the one-time "cheater" cells are essentially forced to display a producer phenotype. However, if competing (incompatible) plasmids arise in a population which do not carry the gene for production of the public good then they can prevent the invasion of producer plasmids (Mc Ginty et al. 2011). Thus the benefits that a plasmid carrying a producer gene gains from infecting other cells are reduced in the presence of incompatible plasmids.

HGT via plasmids can potentially increase local relatedness by infecting previously unrelated neighbours. As relatedness is measured at the locus of interest (which, in this case, is a gene on a plasmid), HGT thus has the potential to increase local relatedness. Nogeuira et al. (2009) proposed that more mobile loci create higher relatedness among their neighbours at those loci by virtue of their mobility, allowing kin selection to maintain costly public goods production. There are therefore two complementary ways in which public goods can be maintained via plasmids: infectious transfer (i.e. spread of plasmids into previously uninfected cells, directly increasing the number of plasmid carriers) and kin selection, where plasmids increase their within-host relatedness ensuring the production cost of public goods is going towards helping relatives.

While recent work suggests that plasmid transfer may not be sufficient to maintain PG in every scenario (Mc Ginty et al. 2011), it is likely that both infectious transfer and kin selection may act as complimentary forces in the evolution of plasmid-borne PG (Rankin et al. 2011a). Infectious transfer of the producer plasmid forces the receiving cell to adopt a producer phenotype, but, when the plasmid is rare, transmission also has the effect of increasing the relatedness between neighboring cells (with respect to the producer gene). Kin selection then maintains PG between relatives. Both these mechanisms could help to explain why so many social genes are transmitted horizontally (Nogueira et al. 2009). There has been some debate as to whether kin selection truly plays a role in maintaining bacterial PG via horizontal gene transfer or, if instead, only the infectivity of the mobile genetic element has an effect (see Giraud & Shykoff 2011; Rankin et al. 2011a) . It has recently been asserted that there is no definitive evidence that kin selection acts, in addition to the infectivity of mobile genetic elements, as a mechanism to maintain bacterial PG (Giraud & Shykoff 2011). Here we aim to examine whether relatedness can in fact be disentangled from infectivity as a process to support plasmid-borne PG. Previous work on plasmid evolution and persistence have drawn on epidemiological models of plasmid transfer (Bergstrom et al. 2000; Lili et al. 2007; Mc Ginty et al. 2011; Rankin et al. 2010; Stewart & Levin 1977), which assume large, well-mixed populations or have not explicitly partitioned the effects of infectivity and kin selection (Smith 2001). Here we build an explicit population genetical model, incorporating horizontal transfer between local hosts. This allows us to examine the relative force of both infectivity and kin selection in the success of plasmid-borne genes. We can, in particular, explicitly calculate relatedness and examine how it is affected by horizontal gene transmission, a

feature that has not been explored by previous models of plasmid-borne PG (e.g. Mc Ginty et al. 2011).

2.3 Model and Results

A model of plasmid-borne public goods production

Life cycle

We assume a population of bacteria living in an infinite number of hosts (an infinite island model), where there are *N* founder strains on each host. Hosts are referred to as "patches" as they represent structure in the population. Generations are non-overlapping, and individuals are haploid. Our model lifecycle consists of five steps:

(1) Founding

Each patch is colonized by *N* independent founder stains sampled from an infinite, panmictic pool of potential founder strains. Founder strains may be plasmid carriers or plasmid-free.

(2) Reproduction

All initial founder cells produce a large number of offspring such that by the end of the reproduction stage there are a very large number of individuals in each patch with a fraction 1/*N* of them descending from each founder strain. Plasmids are inherited vertically from parent to offspring. Parent cells die.

(3) Plasmid transmission

Offspring interact randomly within the patch. Conditional on contact between plasmid carriers and plasmid-free cells in a patch, transmission of the plasmid from plasmid carriers to plasmid-free cells occurs with probability β.

(4) Public goods production

All plasmid-carrying offspring produce a public good, which generates a benefit B that is shared by all individuals within the same patch. The cost of producing the public good to the producer individual is represented by C (this includes the baseline cost of plasmid carriage, where we can write $C=C_C+X$, where C_C is the cost of public goods production and X is the cost of plasmid carriage (Lili et al. 2007)). Offspring survival is determined by results of costs and benefits of public goods.

(5) Dispersal

All cells disperse to form an infinite, panmictic pool of potential founders.

Model Structure

We use a standard population genetical approach, and derive our model from the Price Equation (Price 1970; Price 1972) in order to evaluate the change in the average frequency, p, of the plasmid in the population (Δp, as described formally below).

$$\Delta p = \frac{1}{w}\mathrm{Cov}[w_{ij}, p_{ij}{}^{t}] + \mathrm{E}[\Delta p_{ij}] \qquad [1]$$

where w_{ij} represents the fitness of an individual, *i*, carrying the plasmid, in patch *j*, *w* refers to the mean fitness across the whole population, p_{ij}^{t} is an indicator variable taking the value one if descendant individual *i* in patch *j* carries the plasmid and zero otherwise. (where the subscript *t* indicates it is measured after transmission stage) and Δp_{ij} is the change in an individual's status (plasmid carrier or plasmid free) within a generation. The full derivation of this equation (1) is shown in Appendix A equations (A1-A2). A list of the parameters used in the model is found in Table 1.

Transmission $(\mathrm{E}[\Delta p_{ij}])$

The change in frequency due to transmission, $\mathrm{E}[\Delta p_{ij}]$, is calculated using the life cycle described above. As $\mathrm{E}[\Delta p_{ij}] = \mathrm{E}[p_{ij}^{t}] - \mathrm{E}[p_{ij}]$ and $\mathrm{E}[p_{ij}] = p$ we need only calculate $\mathrm{E}[p_{ij}^{t}]$ which is given by:

$$\mathrm{E}[p_{ij}^{t}] = p^{t} = p + (1-p)\frac{N-1}{N}\beta p, \qquad [2]$$

This equation is composed of the sum of the average population frequency of those who originally carried plasmid (p) plus those non-carriers who were infected with the plasmid ($1 - p$) at the transmission stage. These plasmid-free individuals are infected with probability β by plasmid-carrying individuals that are descended from a different strain to

their own $((N-1)/N)p$, where N is the number of founding strains in a patch. This gives the average frequency of the plasmid in the population after transmission (p^t). Therefore $\mathrm{E}[\Delta p_{ij}] = p + (1-p)((N-1)/N)\beta p - p.$ Thus, we see that the change in frequency after one generation depends on the variance in the population $(p(1-p))$, and a transfer coefficient based on the number of strains and the probability of transfer $((N-1)/N)\beta$.

$$\mathrm{E}[\Delta p_{ij}] = p(1-p)\frac{N-1}{N}\beta. \qquad [3]$$

Selection $\left(\frac{1}{w}\mathrm{Cov}[w_{ij}, p_{ij}{}^{t}]\right)$

We consider a producer plasmid. The fitness of an individual, *i*, carrying the plasmid, in patch *j*, is calculated as

$$w_{ij} = 1 - Cp_{ij}{}^{t} + Bp_{j}{}^{t}.$$

The cost of producing the public good to the producer individual (*i*) is represented by C (this includes the baseline cost of plasmid carriage , where we can write $C=C_C+X$, where C_C is the cost of production of the public good and X is the cost of plasmid carriage (Lili et al. 2007)) and B represents the benefit of producer behavior (shared by all individuals within the same patch *j*). Therefore mean fitness across the whole population, after transmission and fitness interaction, is calculated as:

$$w = 1 + (B - C)p^{t}.$$

$\mathrm{Cov}[w_{ij}, p_{ij}^{t}]$ is calculated as (see Appendix B equation (B1) for full details):

$$\mathrm{Cov}[w_{ij}, p_{ij}^{t}] = \mathrm{Var}[p^{t}](BR - C), \qquad [4]$$

Where $\mathrm{Var}[p^{t}]$ describes the variance in plasmid carriage across individuals and R refers to relatedness; this is a whole-group relatedness coefficient which measures the extent to which random recipients are more (or less) likely to carry the same plasmid as the actor than is an average cell in the patch (Pepper 2000). Full derivations of both $\mathrm{Var}[p^{t}]$ and R are found in Appendix B (equations B2 and B3 and B6 respectively).

Relatedness when the plasmid is rare

The full expression for R is complicated and thus we study special simple cases. First, we consider that the plasmid is rare (i.e. by setting $p \to 0$), in which case we obtain

$$R = \frac{1}{N}\frac{(N + (N-1)\beta)^{2}}{N^{2} + (N-1)\beta^{2}}.$$

For an actor (i.e. a focal cell) in a patch containing N (number of founder strains in a patch) strains, one's own strain makes up $1/N$ of the total set of recipient strains (with whom one interacts), the second component of relatedness is made up of the remaining strains and the effect of horizontal gene transmission. As such, as patch size becomes very large, relatedness due to horizontal gene transfer can no longer build up as a result of fluctuations in transmission between patches. This is a generic feature of our models and is qualitatively equivalent to the decrease in relatedness as patch size increases under vertical transmission with limited dispersal (Rousset 2004; Taylor 1992).

Relatedness around small values of β

Allowing the frequency of the plasmid to vary freely, while assuming that the transmission parameter β is small, we can perform a first order Taylor expansion of relatedness (equation (5)) around $\beta \to 0$, which shows that:

$$R = \frac{1}{N} + \frac{2\big((N-1)(1-p)\big)\beta}{N^2}.$$

HGT will cease to increase relatedness when $p \to 1$ (see Figure 1), and we have $R = 1/N$ so that, when the plasmid is fixed, horizontal gene transmission no longer has an effect as all offspring will receive the plasmid from their respective parent cells.

This is a good approximation of relatedness for values of β up to around $\beta = 0.4$ (not shown). We find that irrespective of the probability of transmission, as the number of founder strains (N) becomes very large relatedness (R) goes to zero. Thus the accumulation of relatedness depends on there being a finite number of founder strains for each patch. This is biologically likely as, for example, many pathogenic bacteria may infect a host starting from a relatively small number of founder cells (FDA 2009), this number may be as low as ten in many cases (placing a severe upper limit on strain numbers). A finite number of founder strains results in a significant variance in plasmid frequency between patches (i.e. hosts) at the founding stage.

Feedback between relatedness and transmission

There appears to be a feedback between relatedness (equation (B3)) and transmission. When p is rare in the population, i.e. close to zero, the variance in founders between patches implies that in many patches there are no plasmid-carrying lineages and only a few patches which are infected by one or two plasmid-carrying lineages. As a result, transmission of the plasmid within a patch will increase plasmid frequency in the patches where there are plasmid lineages but will have no effect on patches from which plasmid lineages are absent. Therefore the variance in plasmid frequency between groups increases and thus whole-group relatedness (R) increases concurrently. On the other hand, when p is close to 1 (close to fixation in the population), the variance between patches implies that there are many patches with N plasmid-carrying lineages and a few patches with N-1, N-2 plasmid-carrying lineages (i.e. which still have a few plasmid-free lineages). Transmission will increase plasmid frequency in the patches not fixed for

plasmids (those which still have plasmid-free lineages) but will not affect the patches within whom the plasmid is fixed. This will decrease the variance in plasmid frequency between patches. Therefore we see a decrease in whole-group relatedness (*R*).

Plasmid spread is affected by relatedness and transmission

Substituting equations (2-4) into equation (1) gives the full expression for the change in the average frequency of the plasmid in the population:

$$\Delta p = \frac{1}{w}\left(\mathrm{Var}[p^{t}](BR - C)\right) + p(1-p)\frac{N-1}{N}\beta. \qquad [5]$$

Substituting equation (B2), and equation (B3), into this expression and performing an invasion analysis (of point $p \to 0$) by taking the partial differential of equation (5) with respect to p and setting p to zero, we find that the plasmid can spread from rare in the population (when $p \to 0$) provided:

$$BR + \beta\frac{N(N-1)}{N^2 + \beta^2(N-1)} > C, \qquad [6]$$

where R refers to relatedness after transmission and $\beta N(N-1)/(N^2 + \beta^2(N-1))$ accounting for the infectivity effect of the plasmid, that is, the increase in plasmid carriage caused by HGT of the plasmid.

Inequality (6) highlights the fact that different types of traits respond differently to being carried on plasmids. When there is a trait which is beneficial to the group (i.e. $B>0$), both kin selection and infectivity influence its spread but for an individually beneficial trait (where $B=0$ and $C<0$), only infectivity plays a role. For traits involved in competition, i.e. those which impact negatively on the group (when $B<0$) and which may be strictly selfish (i.e. $B<0$ and $C<0$), we see that the kin selection effect will hinder their spread. For traits which are parasitic (i.e. $C>0$ and $B=0$), infectivity alone plays a role and this must be greater than the parasite's cost in order for the trait to spread.

Using our expression for relatedness (equation (B3)), we can also calculate relatedness before transmission, which gives $1/N$; the probability of sampling two individuals from the focal strain. Thus we see that when the plasmid is rare the change in relatedness over one generation (ΔR) is calculated by $\Delta R = \frac{N-1}{N}\beta\frac{N(2+\beta)-2\beta}{N^2+(N-1)\beta^2}$. HGT promotes the spread of the plasmid through both ΔR, the additional kin selection effect stemming from the extended identity-by-descent through horizontal spread of the plasmid, and the infectivity effect. Both of these effects are affected by the transmission probability (β) but to different extents as can be seen from Figure 2. It is clear from Figure 2 that the probability of transmission has the greatest impact on the infectivity effect, supporting the assertion based on our Taylor expansion of R around $\beta \to 0$, that the increase in R due to HGT is at most of magnitude $1/N$ when transmission is strong ($\beta \to 1$).

We see that when taking the limit of inequality (6) when N approaches infinity that the kin selection effect drops out leaving only:

$\lim_{N\to\infty} \beta > C$.

This illustrates that there remains an effect of transmission even after the effect of kin select is removed suggesting that under some conditions infectivity may be the dominant component influencing the plasmid's spread. Our results clearly depend on our lifecycle and, if transmission were to occur after the public goods interaction (i.e. if the order of stages 3 and 4 was reversed) then we would no longer see the kin selection effect but infectivity effect would remain.

Inequality (6) reveals that transmission is a powerful force in the model. Even costly plasmids or those which have no effect (i.e. B=0), or even a negative effect, on the group (i.e. $B < 0$) can spread from rare provided transmission is high enough (see Figure 3). However there are other factors, such as relatedness, in the model which are influenced by transmission. Transmission affects relatedness (Figure 4) and we can explore the effects of relatedness on its own by looking at what happens when transmission is rare. If transmission is absent ($\beta = 0$) then we find that equation (5) reduces to

$$\Delta p = p(1-p)\left(\frac{BR - C}{1 + Bp - Cp}\right),$$

where whole-group relatedness, in the absence of transmission, is given by $1/N$. In this case the plasmid can spread from rare provided $BR > C$, i.e. if the producer lineage in patch receives a positive net fitness benefit, the public good will be selected for (a standard result from social evolution theory).

2.4 Discussion

Our model, which is based on an infinite island model, highlights the importance of both infectivity and kin selection as factors enabling PG traits on plasmids to spread through the population. While it has been argued that infection of uninfected cells alone is enough to drive bacterial public goods (Giraud & Shykoff 2011), in a recent study (Mc Ginty et al. 2011), it was shown that this does not apply when there is competition among plasmids in an unstructured environment, and it has been argued that HGT confers a strengthened inclusive fitness benefit to PG in a structured environment, as it increases relatedness on a local scale (Nogueira et al. 2009). However, both infectivity and kin selection are complimentary (Rankin et al. 2011a) and our model helps to reconcile these two approaches. We find HGT favours plasmid-carried public goods through the dual effect of increasing local relatedness when the plasmid is rare and through the effects of transmission (increasing numbers of plasmid carriers).

It is well established that PG can be maintained through interactions between relatives via kin selection (Grafen 1985; Hamilton 1964). It is important to consider that relatedness is always measured at the locus of interest. In the case of our model, the locus of interest is always on a plasmid, and our model shows that, under local transmission, gene mobility can act to increase whole-group relatedness at the plasmid level (see Figure 4). Thus, in the case of plasmid-borne PG genetic relatedness between interacting bacterial cells after the infection period can be generated either through descent from the same founder (i.e. coalescence, which is independent of gene mobility) or through HGT (i.e. transmission of

the plasmid) itself. In the absence of HGT relatedness between cells within the host will be 1/*N*.

It is clear that HGT will increase the number of local cells which carry the plasmid, and thus increase the probability of identity in plasmid carriage, relative to the rest of the population. In the case of plasmid-carried PG, the results of our model show that there is an interesting feedback between transmission and relatedness: if individuals are less related in a patch there will be a higher number of cells for the plasmid to infect which will increase overall transmission whereas if patches are homogeneous a plasmid will never find itself in a patch with uninfected cells. In other words, low relatedness at the plasmid level facilitates plasmid transmission and thus an increase in relatedness. However, high initial plasmid frequency results in less available cells for the plasmid to infect (i.e. decreased transmission). We see that $E[p_j^t p_j^t]$, the probability that two individuals sampled randomly from patch *j* bear the focal allele, continues to increase until the plasmid reaches fixation even when the plasmid is at high initial frequencies (Figure 1). Fixation of the plasmid within a patch means that patch will no longer be affected by transmission. Transmission will subsequently only increase plasmid frequency in the groups not fixed for plasmids (those which still have plasmid-free lineages). This will decrease the variance in plasmid frequency between patches. Therefore we see a decrease in whole-group relatedness (*R*) as the global frequency of *p* increases but we continue to see an increase in $E[p_j^t p_j^t]$ under the same conditions (Figure 1). This aspect of our model is novel because it exhibits a subtle feature of horizontal gene transmission across the population. We expect the increase in the local

frequency of a producer plasmid ($E[p_j^t p_j^t]$) within a patch to favour public goods production. While one may expect to observe such production of public goods associated with the highest relatedness value, at least on a local scale, we demonstrate that a low relatedness value may be associated with success of PG under certain conditions (i.e. that this decreased relatedness is associated with high global frequencies of *p*).

Transmission therefore has two main impacts in plasmid dynamics; firstly via direct transmission gains and secondly via changes to population structure *R*, which modify selection on social traits. The direct gain via transmission (e.g. Giraud & Shykoff 2011) can potentially work in a well-mixed population under certain conditions. However this model has an important limitation, namely that plasmids of the same incompatibility type may exist in the population which do not carry the producer gene (Novick 1987). If two plasmids are incompatible it means that cells cannot carry both plasmids. In this case the advantage of transmission will break down (assuming both plasmids transmit with the same probability). This means that, in a well-mixed population the non-producer plasmid will always invade as it gets a benefit from the producer plasmid (*B*) without paying the cost (*C*). Under such a scenario population structure (i.e. kin selection), and not infectious transmission, is needed for producer plasmids to persist when in competition with non-coding plasmids (Mc Ginty et al. 2011).

The importance of kin selection is further highlighted by the impact that changing the number of founders (*N*) has in our model. Population structure has been found to promote the persistence of public goods production. In our model, the number of founder strains

has two effects. A higher number of founders strains means that producers are more likely to interact with more cheaters and the public good must be shared among more individuals, while a lower number of founders favours the public goods trait, as the offspring of the founders will be more related to each other, within a patch. Increasing relatedness via transmission of the plasmid feeds into this mechanism. However, a second effect of the number of founders is that, when the plasmid is rare, it increases the chance that there are uninfected cells with a plasmid, and thus favours "infectivity" as a mechanism to promote plasmid-borne public goods production. Thus infectivity and relatedness combine to promote public goods. The importance of the transmission term can be seen by the result that a sufficiently high transfer probability can be used to spread a purely costly plasmid which has a negative impact on other individuals (-B) or which has no impact on other cells ($B = 0$) and only incurs a cost to the carrier (Figure 3). In this case a sufficiently high transmission probability allows the otherwise costly producer plasmid to persist. However, the selection term would disfavour a highly selfish and highly infectious plasmid, as the indirect fitness costs of damaging related neighbours would be increasingly severe with increasing transmission.

We assume that our public good is continuously produced by the producer cells and not recycled or regulated by its concentration in the environment. It has recently been demonstrated that such regulation of a durable public good greatly reduces the selection for cheaters in that environment (Kümmerli & Brown 2010). However, as the authors point out, upon invasion of a new patch, the cost of production must be paid in full, at least in this initial period. Thus in this scenario, a high number of founders remains a

threat to public goods production which can be dealt with via an increased transmission probability suggesting the advantage of durable public goods that are facultatively regulated may be maximized when carried on plasmids with relatively high transfer probabilities.

As the size of the founding inoculum is of clinical relevance and can vary for different pathogens (Schmid-Hempel & Frank 2007), this model is useful as it demonstrates the effects of founder size (which can be no greater than inoculum size). When *N* is high, that is, when there is a large and diverse founding inoculum, we see a decrease in the effects of transmission on relatedness (Figure 4) as well as an increase in the transmission bias term. Thus for a high inoculum threshold direct transmission gains, i.e. transmission from carrier cells to plasmid free cells is more important than the effect of transmission on relatedness (and consequently kin selection). However, for a low inoculum threshold the effect of transmission on relatedness is of greater importance and kin selection plays a greater role. We can therefore predict that when inoculum thresholds are low, the plasmids present are more likely to be those coding for public goods than when inoculum thresholds are high (as at low inoculum sizes such plasmids retain the advantage of kin selection).

Plasmids are among the key vectors of HGT, are present in all branches of the bacterial 'tree of life' and have been found in all bacterial communities studied to date (Sorensen et al. 2005). They may act as vehicles for the horizontal transfer of genes between distantly related bacterial species, contributing to bacterial speciation and adaptation

(Ochman et al. 2000). This ability to spread infectiously and reprogram the functionality of host cells may also have potential for use in new medical intervention "Trojan horse" strategies (Brown et al. 2009). More generally, an understanding of plasmids is essential to an understanding of evolution of bacterial traits such as virulence and antibiotic resistance, which have an impact on human health.

In summary, we can conclude that the interaction between relatedness and infectivity is central to a complete understanding of plasmid-borne public goods production and the potential importance of HGT in the spread of producer traits. Plasmids carry a wide range of different genes, but why are so many producer traits mobile (Nogueira et al. 2009)? The results presented here suggest that it is likely that both transmission (directly, on a transient local scale) and relatedness (modified by HGT) play a role in the ecology and evolution of plasmid-borne public goods.

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2.5 Tables

Table 1. List of model parameters

Parameter	Definition
p_{ij}	An indicator variable taking the value one if founder *i* in patch *j* carries the plasmid, zero otherwise, this is a random variable whose value depends on the individual sampled.
p_{ij}^{t}	The value of p_{ij} when measured after transmission (denoted by the superscript *t*). Random interactions indicate this will be $p_{ij}^{\,t} = p_{ij} + (1-p_{ij})\sum_{k\neq i}\frac{p_{kj}}{N}\beta = p_{ij} + (1-p_{ij})\beta p_j$.
p	Average frequency of carriers of the plasmid among individuals in the population such that $p = \mathrm{E}[p_{ij}] = \lim_{n\to\infty}\left[\sum_{ij} p_{ij}/(Nn)\right]$. A subscript *t* denotes when this is measured after transmission.
p_j^{t}	A random variable such that $p_j^{\,t} = \sum_i p_{ij}^{\,t}/N$ and denotes the average frequency of the plasmid in patch *j*. The value of p_j^{t} depends on the sampled individual (descending from founder *i*).
w_{ij}	A random variable indicating the fitness, after transmission, of individual *i* in patch *j*.
w	Average fitness, after transmission, across the population
β	Transmission probability of the plasmid
N	Number of founder strains in a patch

2.6 Figure Legends

Figure 1. As the global frequency of the plasmid increases the within patch pair identity increases until the plasmid goes to fixation but whole-group relatedness decreases. Based on model (a). N =10, β = 0.5. Panel (a) the dotted line indicates where β = 0.5, the broken line indicates relatedness in the absence of horizontal gene transfer i.e. β = 0. Panel (b) β = 0.5.

Figure 2. The effects of relatedness and infectivity are affected by the probability of transmission in different ways.
Dashed lines indicate the infectivity effect, Solid lines indicate ΔR. N = 2 denoted by grey lines and N = 20 denoted by black lines.

Figure 3. Sufficiently high rates of transfer can spread purely costly traits and those that have a negative effect on the population.
C = 0.15, N = 10, run for 10,000 generations. The black area indicates where all cells are infected with the plasmid. The grey area indicates where the plasmid cannot spread and the white area indicates the area where there is coexistence between plasmid carriers and plasmid free cells. This occurs when the plasmid spreads a purely costly trait.

Figure 4. Increasing the rate of plasmid transfer increases the relatedness with a population.
p = 0.001.

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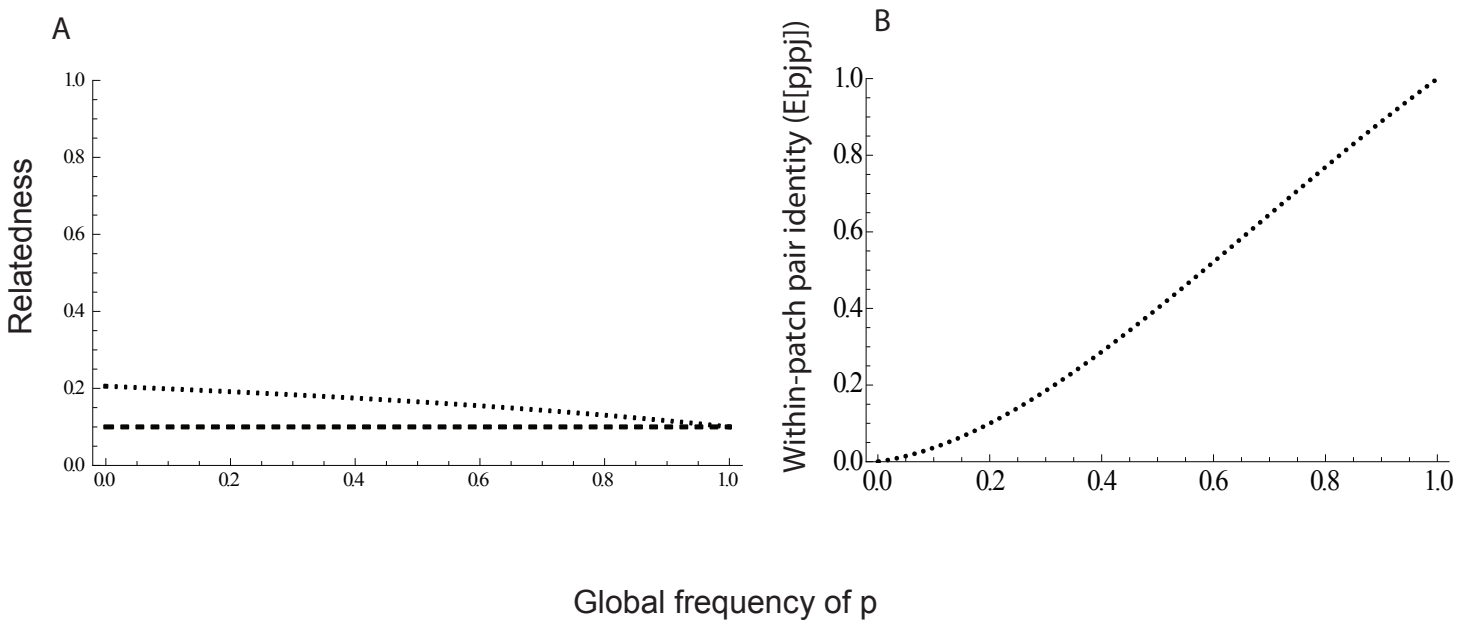
A
Relatedness
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
B
Within-patch pair identity (E[pjpjl])
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Global frequency of p

Figure 1

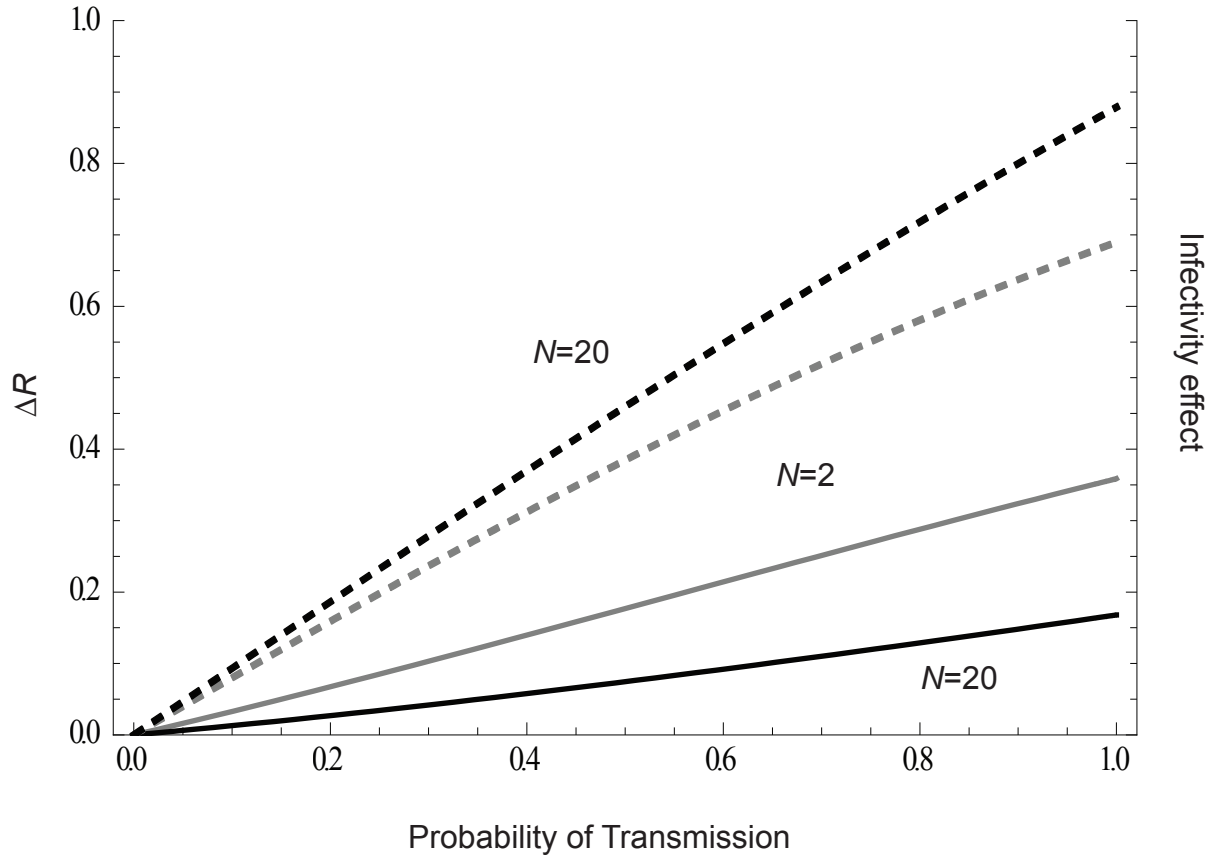
1.0
0.8
0.6
0.4
0.2
0.0
ΔR
N=20
N=2
N=20
0.0
0.2
0.4
0.6
0.8
1.0
Probability of Transmission
Infectivity effect

Figure 2

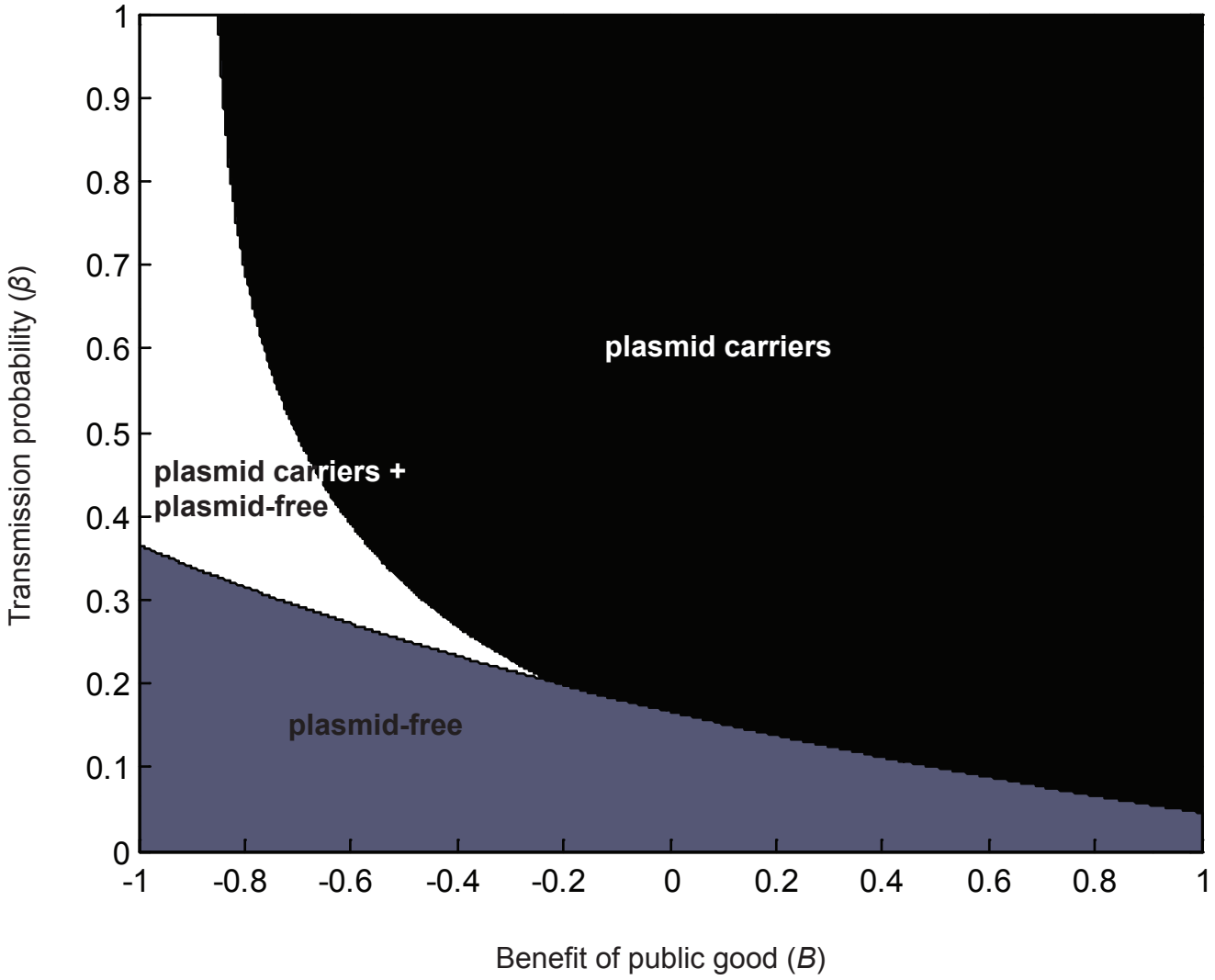
plasmid carriers
plasmid carriers +
plasmid-free
plasmid-free
Transmission probability (β)
Benefit of public good (B)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1

Figure 3

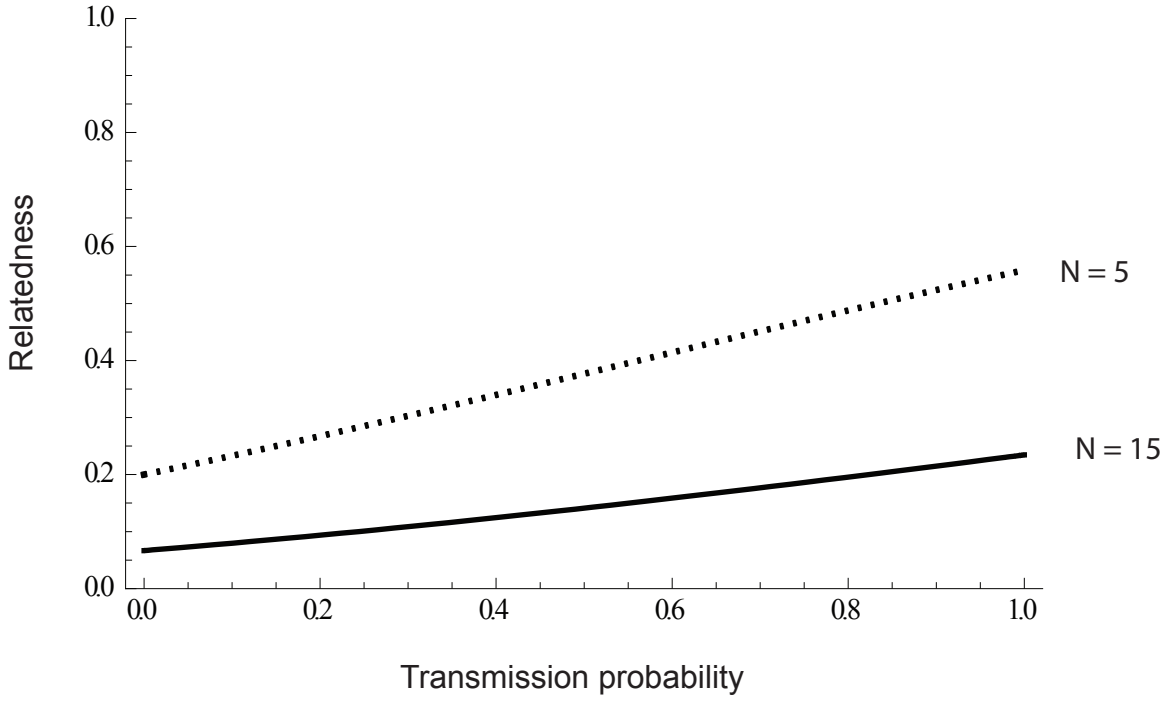
1.0
0.8
0.6
0.4
0.2
0.0
Relatedness
N = 5
N = 15
0.0
0.2
0.4
0.6
0.8
1.0
Transmission probability

Figure 4

3 The Role of Horizontal Gene Transfer and Gene Dosage in the Evolution of the Secretome

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To be submitted

3.1 Abstract

Bacteria display a diverse range of social traits which may be spread through both vertical and horizontal transmission via vectors such as plasmids. Transmission via plasmids may provide many advantages. Firstly, infection by a plasmid allows the spread of costly genes. Secondly, the spread of plasmids can increase local relatedness by increasing the numbers of carriers of social genes within an interacting population. A third advantage to plasmid-based spread comes from the fact that multiple copies of plasmids are frequently carried within a single cell, thus allowing increased expression of plasmid-based genes.

We use genes encoding secreted proteins as a proxy for sociality and, using both theoretical modeling and analysis of bacterial proteins, we investigate the factors which may promote the spread of genes coding for secreted proteins. We find plasmid-based gene duplications are more likely to invade than those based purely on vertical transmission. Genes coding for secreted proteins tend to be more recently acquired and more likely to be carried on plasmids than on the bacterial chromosome. Smaller plasmids, which can have higher copy numbers, tend to carry proportionately more secreted proteins. Additionally secreted proteins tend to be more highly expressed and less costly. Overall our results suggest that horizontal transmission and high product expression through increased gene dosage and gene expression levels may promote the spread of secreted proteins and thus social behaviour among bacteria.

3.2 Introduction

Microbes display a broad range of social traits, ranging from quorum sensing molecules used in communication to the building of diverse protective structures such as biofilms (Crespi 2001; West et al. 2007; Nadell et al. 2009; Rumbaugh et al. 2009). One of the most noted forms of microbial social behavior involves the production of public goods. These are molecules which are secreted into the environment at a cost to the producing cell and confer benefits not only to the secreting cell, but also to surrounding cells (West et al. 2006; West et al. 2007). Many traits associated with virulence or antibiotic resistance involve the production of public goods, for example, the production of nutrient scavenging molecules (West and Buckling 2003) or the release of enzymes to breakdown antibiotics (Ciofu et al. 2000).

Bacteria are able to acquire new genes by horizontal gene transfer (HGT) (Frost et al. 2005). One of the most common forms of HGT is through the transmission of plasmids, which are circular strands of extra-chromosomal DNA that are capable of replicating independently of the host genome (Sorensen et al. 2005; Slater et al. 2008). Plasmids are sometimes viewed as "genomic parasites" as they use host resources for their own reproduction (Rankin et al. 2011b) and often code for genes which are advantageous to their own persistence but disadvantageous to their host, e.g. toxin-antitoxin complexes (Hayes 2003). However, they frequently carry genes which are beneficial to the host cell. Such beneficial traits include those involved in antibiotic resistance (Schumann 2001; Bennett 2008; Svara and Rankin 2011), but also include social traits, such as those involved in bacterial virulence (Nogueira et al. 2009).

Genes that are carried on plasmids benefit from horizontal gene transfer as even genes which are not currently useful to the host can be maintained in the population through horizontal gene transfer (e.g. Bahl et al. 2007). However, plasmid-carried genes also stand to benefit from higher gene expression as a result of gene dosage, where multiple copies of a given plasmid in a cell will increase the frequency of genes (Kondrashov and Kondrashov 2006; Treangen and Rocha 2011), which may, in turn, increase the expression levels of those genes (Nguyen et al. 2006; Hastings et al. 2009). Increased gene dosage may also entail fitness costs (Lenormand et al. 1998; Guillemaud et al. 1999), such as higher metabolic costs (Wagner 2005) or by disrupting the stoichiometric balance of protein complexes (Papp et al. 2003). However, increased gene dosage is often adaptive as, for example, in the case of genes against toxins or antibiotics, where higher gene dosage leads to greater rates of detoxification (Kondrashov and Kondrashov 2006; Andersson and Hughes 2009; Sandegren and Andersson 2009).

Social traits are frequently gained and lost in bacterial populations (Nogueira et al. 2009). Much of this gain involves horizontal transfer of the genes that encode the trait (Smith 2001), and it has previously been shown that secreted proteins are over-represented on plasmids (Nogueira et al. 2009). Plasmid carried social traits provide several advantages from the view point of social evolution. Firstly, plasmid transmission can allow costly traits to be maintained through HGT, which would benefit social traits, which are costly to the individual (Smith 2001). Secondly, plasmid transfer can influence local associations between cells by altering local population structure, which will favour cooperative traits through kin selection (Nogueira et al. 2009; Mc Ginty et al. submitted). Thirdly, because a single cell can carry multiple copies of a plasmid, multiple copies of a plasmid can increase gene dosage (del Solar et al. 1998; Friehs 2004). Genes transferred horizontally generally tend to be poorly expressed

(Thomas and Nielsen 2005; Lucchini et al. 2006), but plasmid-borne genes may alleviate this problem due to the fact that a plasmid can have a high copy number inside a cell. Because high expression of a gene product encoding a cooperative trait may be associated with efficient cooperation (Harrison et al. 2006), high gene dosage may increase this efficiency. As public goods are secreted into the environment, high expression, driven by increased gene dosage, may provide increased direct benefits from cooperative behavior.

Here we investigate the role of gene dosage in the evolution of social traits. Specifically, we test the hypothesis that some social traits are carried on plasmids because plasmids confer high gene dosage, and thus high gene expression, which increases the benefits of cooperation. We note that this benefit of plasmid-borne social traits may act synergistically with the other two benefits, the ease by which plasmids can transmit costly traits through the population, and the alteration of population structure in favor of public goods producers. We use secreted proteins and the genes encoding them as a proxy for social traits. Secreted proteins have been shown to be costly, and recently acquired in their host cells (Nogueira et al. 2009). Additionally, as secreted proteins are released outside of the cell, they may be utilized by other cells in the neighbourhood, suggesting that they should play a role in microbial sociality (Rainey and Rainey 2003; Nogueira et al. 2009). To examine this, we firstly build a model to compare chromosomal-based gene duplications to plasmid-based HGT as a mechanism for increasing gene dosage of cooperative traits. Secondly, we look at the genome sequences of 291 gram-negative bacteria to investigate the role that gene dosage plays in the evolution of secreted proteins. These two approaches allow us to examine how bacterial social behavior may be promoted through HGT of plasmid-borne genes, and through gene dosage effects.

3.3 Model and Results

Here we describe a model to compare the success of two different means by which gene dosage can be increased, namely duplication of chromosomal genes and plasmid transfer. We assume that individuals carry a 'cooperative gene', that is, a gene encoding a public good and we model the case where individuals can either carry one copy of the gene, or two copies of the gene. Cells carrying a single copy of the cooperative gene pay an individual metabolic cost c to produce and secrete a public good. Depending on the type of public good considered, a wide range of benefits may be obtained from these molecules. These include detoxification, such as when enzymes break down antibiotics and other toxic molecules in the environment (Ciofu et al. 2000); nutrition, for example by iron scavenging siderophores (West and Buckling 2003), or host invasion, as for microbial toxins that break down host tissue (O'Loughlin and Robins-Browne 2001). We denote the benefit derived from the public good as b. For cells which carry two copies of the cooperative gene, we assume that the extra gene will increase the production of public goods, and thus generate a supplemental benefit bx (meaning that they produce $b+bx$ of the public good, where $x>0$ with less than a doubling of public good production if $x<1$). At the same time, these cells incur a supplemental cost of cy, meaning they will pay an individual cost $c+cy$, where $y>0$. Cells that carry the second copy of the gene on a plasmid incur an additional individual cost of v, which can be thought of as the expense of using host resources to replicate extra plasmid DNA (Lili et al. 2007). This cost will be amplified depending on the copy number of the plasmid. The parameters we used are also listed in Table 1.

We assume a population of bacteria living in an infinite number of hosts (an infinite island model (Wright 1931)), where there are *n* founding bacterial strains, each with a particular genetic background, on each host. Generations are non-overlapping, and individuals are haploid. The lifecycle is as follows: (1) All cells of the initial strains produce a large number of offspring; (2) plasmid transmission occurs with probability β, in the case that the allele is carried on a plasmid, and is followed by (3) public good production by all cells (sum of public good produced by all individuals, which is determined by the number of copies of the gene) ; (4) all individuals migrate, and finally (5) hosts are re-colonised by offspring chosen randomly from the whole population according to offspring fitness, i.e. offspring compete to found new patches.

Allowing for the spread of the focal gene by horizontal transfer, the condition for a cell carrying two copies of the cooperative gene to invade a population of cells only carrying a single copy of the gene is (for details of the derivation see Appendix A):

$$bx\frac{(n+\beta(n-1))^2}{n^3+(n-1)n\beta^2}+\frac{\beta(n-1)}{n+\beta(n-1)}>cy+v. \qquad [1]$$

In the absence of horizontal transmission (i.e. β =0 and v=0), that is, if the second copy of the gene arises through a gene duplication, inequality (1) reduces to $bx/n>cy$, which can be rearranged to give $bx/cy>n$. This means that the ratio of the marginal benefit to the marginal cost from an extra gene copy must exceed the number of founding strains. Because we assume that a single copy of the cooperative gene is able to persist (meaning that $b/n>c$, and thus $b>c$), this requires that $bx\geq cy$. In other words, the supplemental amount of the public

good produced due to extra gene copies must exceed the supplemental cost. This is an intuitive result, and it demonstrates that the successful invasion of a cooperative gene that originated in a gene duplication is constrained by the conditions that allowed for the invasion of a chromosomally based cooperative trait in the first place.

If the second copy of a gene can be transmitted both horizontally and vertically, then this second copy is more likely to invade. This can be seen from inequality (1), where the conditions to meet the inequality become less stringent if $\beta > 0$. This holds as long as the cost v of plasmid carriage is relatively small. The carriage of a plasmid, in the absence of selection for its genes, can reduce growth by up to ten percent (Simonsen 1991) but this cost is known to decrease over time due to changes in both bacteria and plasmids (e.g. (Dahlberg and Chao 2003; Dionisio 2005). Therefore, in contrast to the previous scenario of exclusively vertical transmission, the invasion of a horizontally transmitted gene copy is more likely than that of a chromosomal copy. Thus, if the cost of carrying two genes is small relative to the additional amount of public good produced, multiple copies of the cooperative gene are more likely to invade if they can be transmitted horizontally on plasmids than if they are encoded on the chromosome. This observation also applies if our invading gene is a modifier of expression, increasing the expression level of an already existing public good gene by producing bx more goods at an additional cost cx.

Based on the observations from our model, we make a number of predictions. (1) As plasmid-transmission is advantageous to social traits, we predict that social genes are more likely to be found on plasmids. (2) As plasmid carried traits tend not to be part of the core genome and instead are generally recently acquired, we would expect social genes to have higher

representation among recently acquired genes than core genes (3) Inequality (1) also demonstrates that social traits are more likely to spread if they are low in cost. Thus we predict that the biosynthetic cost associated with the synthesis of the products of social genes is lower than for genes whose products are not involved in interaction with other cells. Our model suggests that while plasmid-based gene duplications are more likely, chromosomal duplications of social genes are both possible and beneficial. (4) We therefore expect that any social genes encoded on the chromosome will have a high copy number. (5) The advantage of plasmid-spread in increasing gene dosage predicted by our model leads us to hypothesize that in order to maximize this effect, social genes will be carried on high copy number plasmids. (6) We can make an additional hypothesis that does not stem directly from our model but is based on our argument that increased expression aids cooperation and predict that social genes are highly expressed. In the following section we analyze bacterial genomic data to validate these predictions.

To test our model, we inferred protein localization of protein sequences longer than 30 amino acids from 291 strains of gram-negative bacteria that fall into 30 genera. Previous work has shown that secreted proteins i.e. proteins expressed in the extracellular environment can serve as a proxy for social traits (Nogueira et al. 2009). We use the same approach here and describe those proteins whose localization has been designated as extracellular (see Methods section for details) as putative social traits. We determined the ancestry of these proteins using a hierarchical clustering algorithm to group orthologs (see Methods for ortholog identification) into clusters of closely related proteins, whose members have greater than 80 percent sequence identity. Genes within such a cluster have also been called ‘equivalogs’ to indicate that they are more than just orthologs (Nogueira et al. 2009). Because of their high similarity, equivalogs are likely to have similar functions and subcellular localization

(Nogueira et al. 2009). We distinguish between three kinds of equivalog clusters. *Ancestral* equivalog clusters contain orthologous genes across all organisms in a given genus *and* its outgroup. *Core* equivalog clusters contain orthologous genes in every organism of the genus. *Unique* equivalog clusters are clusters of orthologous genes that are neither ancestral nor core and thus contain the most recently acquired genes (Nogueira et al. 2009). Genes contained in these clusters are said to have the ancestral background of their respective clusters (e.g. core equivalog clusters contain genes of core ancestry).

Secreted proteins are plasmid-borne, recently acquired and less costly.

We first asked whether secreted proteins are more likely to be encoded on plasmids than on the chromosome than expected if genes for secreted proteins were distributed randomly across chromosomes and plasmids. We found this to be the case ($\chi 2$= 393.35, df = 1,p-value <0.0001). Our second model prediction is related to the predilection of social genes to be plasmid-encoded. As plasmid genes do not generally form part of the core genome due to the nature of their frequent loss and gain and as social traits are frequently recent adaptations we would then expect that genes encoding social traits would be more likely to be recently acquired. This is indeed the case as such genes are preferentially unique to a genus (see Table 2), and they are less likely to be core or ancestral genes than expected by chance alone (χ-squared= 2384.133, df = 1, p-value <0.0001; χ-squared = 447.3378, df = 1, p-value <0.0001; χ-squared = 1651.724, df = 1, p-value <0.0001; respectively). These observations confirm the first two predictions of the model: that social traits are more likely to be encoded on plasmids and are more likely to be recently acquired.

As our model demonstrates that social traits are more likely to spread if they are low in cost, we investigated whether specific cellular compartments are enriched for cheap or expensive

proteins. The results, shown in Supplementary Figure 1, demonstrate that secreted proteins are the cheapest proteins. This pattern has been shown for a smaller data set before in *Escherichia coli*, among other organisms (Nogueira et al. 2009; Smith and Chapman 2010). It is exactly what theory would predict: If social behavior is easiest to maintain when its costs are low, natural selection should lower the cost of proteins involved in cooperative behavior. Low cost may be especially important for secreted proteins encoded on plasmids as plasmids entail a virulence cost of their own.

Genes encoding social traits have higher copy numbers when encoded on chromosomes. An alternate means of ensuring high production of a protein, as opposed to high expression of individual genes, is to have multiple copies of these genes allowing production of a larger number of transcripts per unit of time (Hastings et al. 2009). Since equivalogs generally share function and subcellular localization (Nogueira et al. 2009), we can identify an effective copy number of genes that encode proteins with a given function by counting the number of sequences per equivalog cluster for each species. Clusters containing more than one sequence are described as multigenic clusters. Figure 1 shows how this proxy of gene copy number depends on subcellular localization, as well as on whether genes are encoded on plasmids (Figure 1(a)) or on chromosomes – Figure 1(b)). On average 3.67 ± 0.02% of chromosomally-encoded secreted proteins come from multigenic clusters, a number significantly higher (χ-squared = 262.478, df = 1, p-value <0.0001) than for non-secreted chromosomally-encoded proteins. Remarkably, this holds only for secreted proteins encoded on the chromosome (Figure 1(b)), but not on plasmids (χ-squared = 4.8604, df = 1, p-value = 0.02748), where secreted proteins come from multigenic clusters in only 2.26 ±0.19% of cases (Figure 1 (a)). We speculate that this difference stems from the fact that plasmids often have multiple copies

and can thus achieve high expression of secreted proteins in this way rather than through an increase in gene copy number.

The relationship between replicon size and social traits

A potential advantage for a gene encoded on a plasmid is the associated increase in copy number relative to the same gene encoded on the chromosome. Naturally occurring plasmids can range in copy number from one to several hundred per cell (Novick 1987) but smaller plasmids tend to have higher copy numbers (Smith and Bidochka 1998; Diaz Ricci and Hernández 2000; Zhong et al. 2011; Ribeiro et al. 2012). For our next analysis we explore whether smaller plasmids are more likely to carry genes encoding secreted proteins. In order to exclude large, non-mobilizable plasmids which act as secondary chromosomes (Smillie et al. 2010), we examined only plasmids of size smaller than 1Mbp. For these plasmids, we analysed the relationship between replicon size and the proportion of genes that encode secreted proteins. We compared this relationship to the pattern obtained for chromosomally encoded secreted proteins (for chromosomes of a size exceeding 2Mbp, also to avoid potential overlap with secondary chromosomes of plasmid origin). Figure 2 demonstrates that the proportion of the total number of genes encoding secreted proteins is larger for smaller plasmids. We find that secreted proteins make up a higher proportion than expected of the genes carried on plasmids of size smaller than 0.1Mbp when compared with larger plasmids (of size smaller than 1 Mbp but larger than 0.1Mbp) (χ-squared = 441.589, df = 1, p-value <0.0001). This pattern is not seen for chromosomal proteins (see Supplementary Figure 2). This suggests that the dosage of genes encoding social traits may be increased by being carried on a small plasmid with potentially high copy number. We also find that conjugative plasmids are less likely to carry secreted proteins (χ-squared = 25.2246, df = 1, p<0.0001) than non-secreted proteins but the opposite was true in the case of mobilizable plasmids (χ-

squared = 34.193, df = 1, p<0.0001), which tend to be smaller in size than conjugative plasmids (Sota and Top 2008). Therefore we see an overall trend towards smaller plasmids being more likely to carry, and carrying proportionately more, secreted proteins than larger plasmids. As smaller plasmids generally have higher copy number our analysis indicates that secreted proteins are preferentially carried on high copy plasmids which allows the maximization of dosage of genes for secreted proteins. As we have previously described, we expect high gene dosage (and associated gene expression) to be beneficial for social traits through increasing the efficiency of cooperation and compensating for any shortfall in the expression levels of horizontally acquired genes.

Secreted proteins are highly expressed.

Additionally, our dataset allows us to examine aspects of secreted protein expression beyond the scope of our model. Cooperative public goods are among the proteins secreted into the environment by bacteria. We hypothesized that higher gene expression (thus more abundant production of public goods) may boost the success of cooperative individuals through the potential increased direct benefit associated with more abundant public goods. Thus, in our next analysis we investigated whether secreted proteins may be more highly expressed than expected by chance alone, as indicated by a higher codon adaptation index (Sharp and Li 1987; Ghaemmaghami et al. 2003; Lithwick and Margalit 2003) of the genes encoding them. We found that among chromosomally-encoded and recently acquired proteins, secreted proteins are associated with a significantly higher CAI than other proteins (with an observed to expected ratio of 1.63 for highly expressed secreted proteins, χ-squared = 233.57, df = 1, p-value < 0.0001). Importantly, one might argue that chromosomally encoded recently transferred proteins, among which extracellular proteins are over-represented, have CAI values lower than expected from their expression patterns because their CAI evolved in

genomes different from the one we analyzed them in. If this problem were to confound our analysis, we would expect a pattern opposite to the one observed, i.e. extracellular proteins should have lower predicted expression levels. Our observations suggest that chromosomally-based secreted proteins, which cannot rely on multiple plasmid copies to increase product formation, may experience selection to improve their codon usage to ensure high expression.

Summary

Our results show that genes for social traits are more likely to be recently acquired and are disproportionately found on plasmids, particularly those which are smaller in size. Genes for social traits are highly expressed and come in higher copy numbers on the chromosome. Additionally, secreted proteins are associated with a low biosynthetic cost. These results indicate that social traits are potentially associated with high gene dosage and expression, and that this may improve the efficiency of their social interaction.

3.4 Discussion

We modeled the role of gene dosage in the evolution of social behaviour. Specifically, we compared two different means by which an increase in gene dosage can come about – plasmid transfer or gene duplication – and the likelihood with which it can invade a population. We found that plasmid-based gene duplications were more likely to invade than those based solely on vertical transmission. We predicted that increased gene dosage is beneficial to cooperative traits and we therefore expected to find that secreted proteins were more likely to be carried on plasmids, particularly high copy number plasmids; that genes for secreted proteins would be recently acquired; that secreted proteins would be associated with low costs

to minimize the metabolic expense associated with cooperation; that genes for secreted proteins would be highly expressed; and that chromosomally-encoded genes for secreted proteins would be found in multiple copies on the chromosome.

Our results suggest that plasmid-based traits are more likely to invade than chromosomally-encoded duplications. This is because their persistence is boosted infection and kin selection advantages stemming from horizontal gene transfer (HGT), which in turn leads to less stringent invasion conditions. This relative advantage of plasmid-borne genes is likely augmented by the fact that gene duplications, while common, are also lost at a high rate. For instance, tandem gene duplications can be lost at rates as high as 0.15 per cell generation, whereas the rate of plasmid loss through segregation is generally on the order of <0.001 per cell generation (Modi and Adams 1991; Simonsen 1991; Sandegren and Andersson 2009). Our model therefore predicts that HGT provides an invasion advantage for social traits. The role of HGT in bacterial social behaviour has been discussed previously (Giraud and Shykoff 2011; Rankin et al. 2011a) with HGT suggested as a novel means by which cooperation may be maintained in populations (Smith 2001). It has previously been shown in *Escherichia coli* that social traits are disproportionately carried by mobile elements (Nogueira et al. 2009). Here, through our bioinformatics analyses, we find support for this claim, with social traits more likely to be carried on plasmids as well as being more likely to be of recent ancestry, which suggests recent mobility.

Although our model suggests that plasmid-based gene duplications are more likely, we see that chromosomal duplications of social genes are both possible and beneficial. Therefore we predict an advantage for social traits associated with gene dosage. We extended our analyses

to examine the basis for our model prediction that plasmids favour increased gene dosage of social traits. If gene dosage provides an advantage for social traits then we would expect that secreted proteins are not only preferentially plasmid-encoded (as we found them to be), but are also encoded by genes with higher copy number when they are chromosomally encoded. Our analysis confirmed this prediction. In contrast, plasmid-carried secreted proteins were not encoded by the most high copy number genes. We suggest that this disparity between the copy numbers of genes for plasmid- and chromosomal-encoded genes stems from plasmid copy number which can increase gene dosage. All plasmids, even those which encode only genes for their own propagation, have a characteristic copy number and initiate and control replication within host cells in order to reach it (del Solar et al. 1998). This makes high gene copy numbers on individual plasmids unnecessary. In contrast, to increase gene dosage on a chromosome, multiple chromosomal copies of the gene are necessary. This supports our prediction that gene dosage effects favour social traits.

If plasmid copy number plays an important role in promoting horizontally transferred social traits than we would further predict that genes for secreted proteins should be preferentially encoded by high copy number plasmids to maximize the gene dosage advantage of horizontal transfer. Plasmid copy number varies extensively, but smaller plasmids are more likely to come in higher copy numbers than larger plasmids (Smith and Bidochka 1998; Diaz Ricci and Hernández 2000). We find a relationship between the size of plasmids and their propensity to encode secreted proteins where smaller plasmids tend to contain a larger proportion of genes that encode secreted proteins. This relationship is absent in chromosomally encoded secreted proteins supporting our hypothesis that genes for secreted proteins are over-represented on plasmids (relative to proteins with alternative localisations) due to the gene dosage advantage that plasmid provide. Because they do not carry their own genes for conjugation, mobilizable

plasmids tend to be small (Sota and Top 2008) and thus mobilizable plasmids are expected to have higher copy numbers. When we compare the genes encoded by mobilizable and conjugative plasmids, we find that mobilizable plasmids are more likely to carry genes for secreted proteins. This lends further support to our hypothesis that smaller, and therefore potentially higher copy number, plasmids carry a disproportionate number of genes for secreted proteins.

We also find that secreted proteins tend to be among the cheapest and most highly expressed proteins. Low costs for secreted proteins are to be expected, because, first, individuals do not necessarily benefit directly from the molecules they themselves secrete, and, second, according to Hamilton's rule, low-cost cooperative traits can spread more easily (Hamilton 1964). Plasmid-carried genes may initially face a disadvantage upon infection of a host cell, because they may not be attuned to the codon usage of their hosts. This may result in less than optimal expression of plasmid genes, including those encoding social traits. However, this low expression may be compensated for by the increased gene dosage of multiple plasmid copies before long term evolutionary adaptation to host codon usage leads to increased efficiency of expression.

There are several limitations to our study. First, information about the function of the genes we study is limited. Because many of these functions are unknown or merely predicted, these genes are best considered to encode putative social traits. We note that in comparison to previous work (Nogueira et al. (2009)) we apply a different criterion to identify such genes. Specifically, we include only genes encoding secreted proteins, rather than genes encoding both secreted proteins and outer membrane proteins. Outer membrane proteins interact with

the environment, but because they are anchored to a host cell, they are less likely to benefit other individuals. Second, we have no information on the copy number of the plasmids we find. Because copy numbers can vary dramatically depending on a variety of factors, such as plasmid size and the environment, it is clear that further research is required to validate the copy number hypothesis. Third, our measurement of protein cost is based purely on amino acid synthesis cost, and does not consider translation costs, the effects of limiting nutrients or other factors that may affect the true of cost of producing a protein

It has been argued that horizontal gene transfer can benefit social traits through the infection of uninfected cells (by increasing the frequency of carriers of cooperative genes) and through the modification of population structure (by increasing the local frequency of cells with the cooperative gene, and thus enabling cooperators to interact more with other cooperators in the surrounding environment) (Smith 2001; Nogueira et al. 2009; Giraud and Shykoff 2011; Rankin et al. 2011a). The relative merits of these effects are discussed in depth elsewhere (e.g. Giraud and Shykoff 2011; Rankin et al. 2011a). Our results suggest that there is a third advantage for social traits carried on plasmids, that of gene dosage and its associated increase in expression. Plasmid transfer allows for cells to carry multiple copies of a gene through plasmid copy number, and thus to potentially increase the expression of that gene. Our results indicate that plasmids that have high copy numbers (such as small plasmids and mobilizable plasmids) will be preferential vectors for genes for secreted proteins, and, by extension, genes for cooperative interactions such a public goods production. Higher gene dosage ensures that horizontally transferred cooperative traits will not lose out in terms of expression levels, as well as well as benefiting from infectivity and increased interactions with other cooperators. Plasmids have been suggested as means to disseminate and maintain easily-exploitable traits

such as public goods production (Smith 2001). Our results suggest another possible advantage to support plasmid carried social traits.

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3.5 Methods

We analysed genomic information of protein sequences longer than 30 amino acids from 291 strains of gram-negative bacteria that fall into 30 genera. We obtained this information from the National Center for Biotechnology Information (NCBI) (Geer et al. 2010). The total dataset we used comprises 1,043,170 proteins. For each protein sequence in the dataset we predicted its cellular localization using the software psort-b (Yu et al. 2010). Psort-b uses a support vector machine trained on sequences with empirically known subcellular localisations to predict where one or more proteins of interest are localized. It distinguishes between extracellular, periplasmic, outer membrane, cytoplasmic membrane and cytoplasmic localizations in gram-negative cells. Supplementary Table 1 summarizes these predictions. Previous work has shown that secreted proteins i.e. proteins expressed in the extracellular environment can serve as a proxy for social traits (Nogueira et al. 2009). We use the same approach here. It was possible to calculate known and singular localisations for 696,339

proteins with known and singular localizations and all subsequent analyses were based on this data.

Energetic costs of social traits

The synthesis of any protein represents an investment for a cell. Secreted proteins represent a greater investment, as they are lost to the environment, meaning their amino acids cannot be recycled and incorporated into newly translated proteins. Evolution is therefore expected to minimize the investment into extracellular proteins to reduce their synthetic burden on the cell (Smith and Chapman 2010). To estimate the energetic cost to synthesize each of our study proteins we used data from Akashi and Gojobori (2002). These authors estimated amino acid biosynthesis cost as the number of ATP molecules needed for the synthesis of each of the 20 natural amino acids. The total cost of a protein sequence is then obtained by summing up the cost of each of its constituting amino acids. We then divided this cost by the protein's length to arrive at a per-amino-acid cost, which takes into account differences in protein lengths.

Expression of social traits

The Codon Adaptation Index (CAI) is a measure of the efficiency of translation which correlates with gene expression (Sharp and Li 1987; Ghaemmaghami et al. 2003; Lithwick and Margalit 2003). We calculated CAI values following Sharp and Li (1987). First, we categorized genes into ancestral, core and recent ("unique"). For our analysis of codon usage bias, we only focus on recent genes to allow us to compare genes coding for proteins at different localizations, while controlling for age of proteins within a given genome. For each of the 30 genera we study (and a suitably chosen outgroup in each genus, see Supplementary

Table 2), we use Blastp for an all-against-all genome-wide comparison of coding regions among organisms within a genus. We define two genes as orthologs if they are best unique reciprocal blast hits with at least 80% amino acid sequence similarity and if they differ by less than 20% in length. The core genome, consisting of genes ubiquitously found among all strains in the genus, was defined as the pairwise intersection of all lists for each genus (Touchon et al. 2009).

In our calculation of codon usage, we derived tables of relative synonymous codon usage from ribosomal proteins of each organism. We used the Codon Adaptation Index (CAI) value to identify proteins that are likely to very highly expressed. Specifically, we designated those proteins whose CAI values were in the top 5% of the CAI value distribution as highly expressed. Using this threshold we examined whether secreted (i.e. extracellularily localised) proteins are more often highly expressed than expected than if highly expressed genes are evenly distributed throughout the different localization categories. The CAI of a particular gene is highly influenced by the match (or mismatch) between the gene itself and the genetic background of the cell in which it is found. We focused only on those proteins which are encoded on the chromosome, as we assume that the CAI of plasmid-coded proteins is unlikely to be comparable due to their mobility. For this analysis we examined proteins that were unique, i.e. recently acquired proteins, among which social traits are more likely to be found (Nogueira et al. 2009).

3.6 Tables

Table 1. Parameters used in gene duplication model

Parameter	Description
n	Number of founding strains
b	Shared benefit of public good
c	Individual cost of public good production
β	Probability of transfer of plasmid
v	Cost of plasmid carriage
x	Moderator of additional benefit produced by multiple gene copies of the gene encoding the public good
y	Moderator of additional cost of expression of multiple copies of the gene encoding the public good

Table 2. Distribution of genes in unique, core and ancestral genes clusters by localization. Percentages of genes for extracellular, cytoplasmic, cytoplasmic membrane, outer membrane and periplasmic proteins which are distributed in equivalog clusters among the three ancestry types (ancestral, core and unique (recently acquired)).

Localisation	Ancestry (%)		
	Ancestral	Core	Unique
Extracellular	2.554843	6.409678	91.03548
Cytoplasmic	19.41141	14.35536	66.23323
Cytoplasmic Membrane	14.04972	12.00998	73.9403
Outer Membrane	8.831446	7.836067	83.33249
Periplasmic	14.05625	10.64958	75.29416

3.7 Figures Legends

Figure 1. Chromosomal genes that encode secreted proteins are more frequently part of multi-gene clusters than plasmid-borne genes. Horizontal axis: C: cytoplasmic, CM: cytoplasmic membrane, E: extracellular, OM: outer membrane and P: periplasmic. Vertical axis: The average percentage of genes which come from multi-gene clusters (i.e. have a copy number >1, genes within the same multi-gene cluster may also be described as paralogs). Bars indicate standard errors of the mean. (a) Plasmid-encoded proteins. (b) Chromosomal-encoded proteins.

Figure 2. The relationship between plasmid size and the proportion of secreted proteins on a given plasmid, showing that smaller plasmids encode a higher proportion of secreted proteins. The horizontal axis shows plasmid size in mega base pairs (Mbp), the vertical axis proportion of genes on a plasmid that encode secreted proteins. Solid lines represent the predictions of a binomial general linearized model, showing the relationship between the proportion of genes coding for extracellular proteins and plasmid size. Broken lines indicate 95% confidence intervals for this model.

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Figure 1

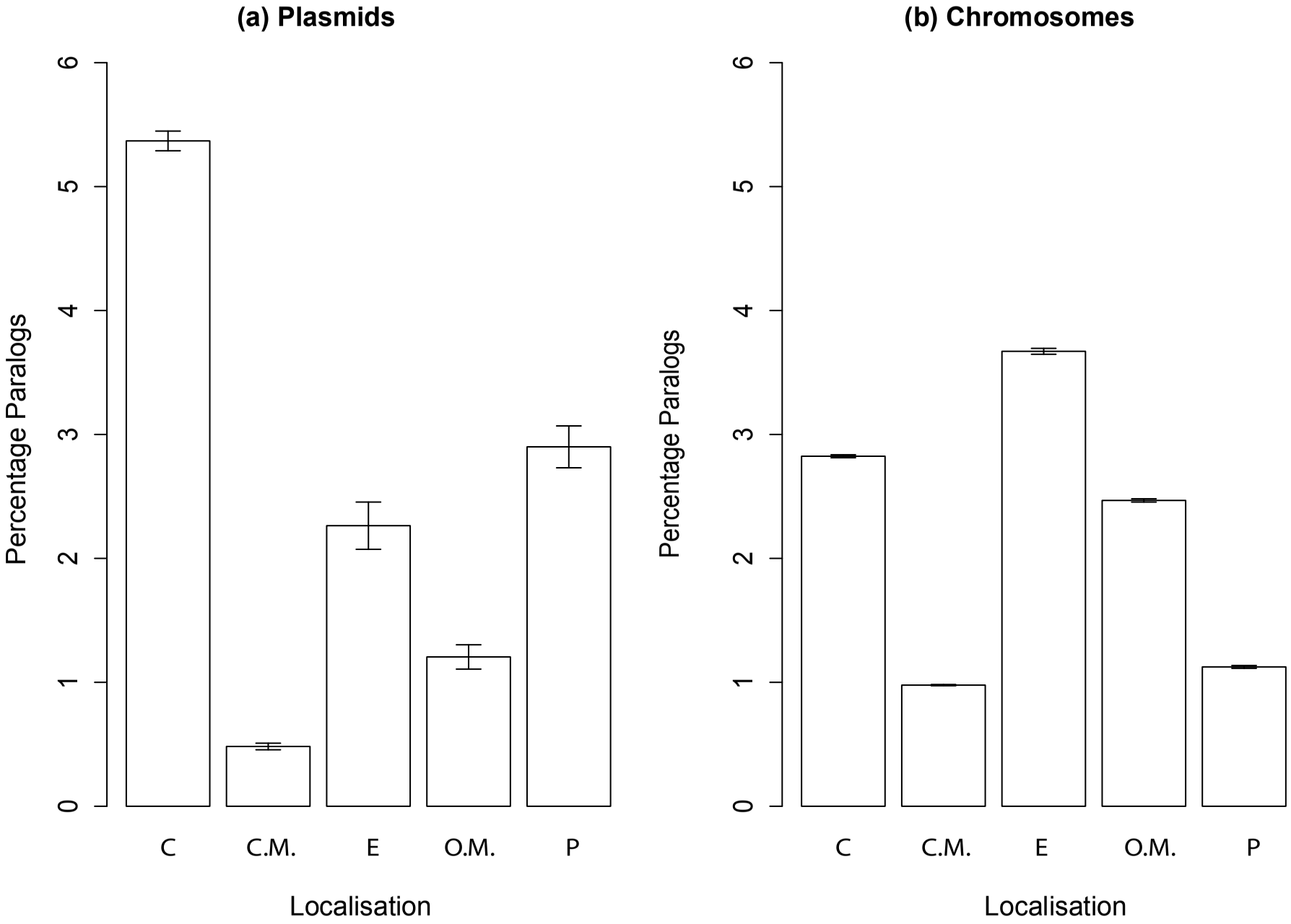
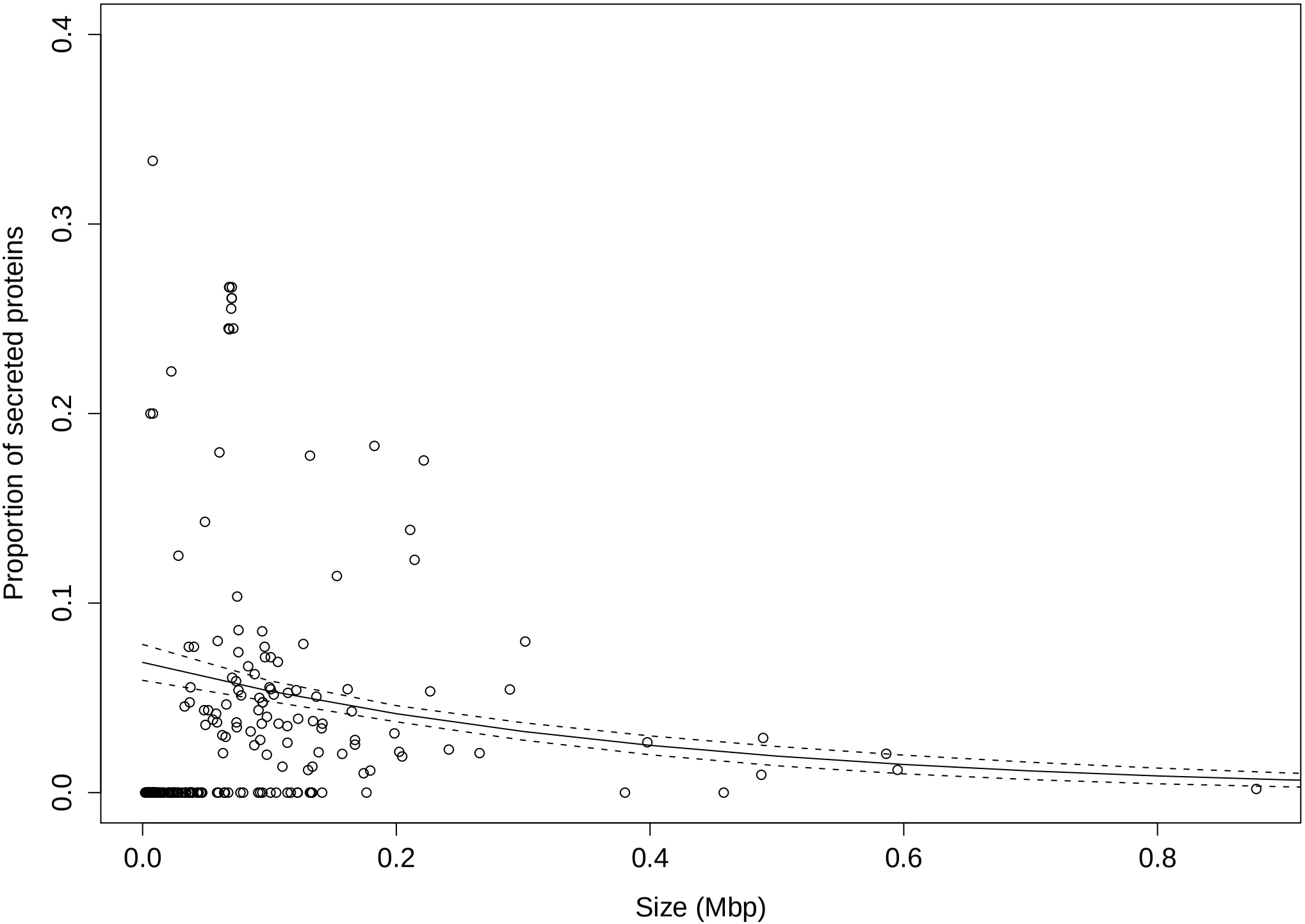

(a) Plasmids
Percentage Paralogs
0
1
2
3
4
5
6
C
C.M.
E
O.M.
P
Localisation
(b) Chromosomes
Percentage Paralogs
0
1
2
3
4
5
6
C
C.M.
E
O.M.
P
Localisation

Figure 2


Proportion of secreted proteins
0.0
0.1
0.2
0.3
0.4
0.0
0.2
0.4
0.6
0.8
Size (Mbp)

4 Horizontal Gene Transfer Promotes the Evolution and Spread of Bacteriocins

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4.1 Abstract

Bacteriocins are an important part of bacteria's diverse anti-competitor weaponry. However, such traits are costly and can struggle to invade a population when rare. When producers are rare there may not be enough toxin to impact sensitive, competing cells, and so they may not obtain any direct benefit from the costly production of bacteriocins. This limitation has been described as a rarity threshold. Bacteriocins are frequently carried on plasmids and here we examine why this means of gene transfer may be advantageous for bacteriocins.

We show that horizontal transfer of the bacteriocin trait via plasmids enables it to successfully overcome the rarity threshold and become established in the population. We find that at this point horizontal transfer is no longer required for the trait to remain stable. However the bacteria are now addicted to the plasmid, in the sense that if any cell loses the plasmid they will be killed by the toxin because the plasmid also encodes an anti-toxin which neutralizes the bacteriocin.

Horizontal gene transfer allows bacteriocins to spread in a population and bacteriocins can allow plasmids to persist even after horizontal transfer is not necessary for the maintenance of the bacteriocin itself. This implies a mutually-beneficial interaction between the traits carried on plasmids and the plasmids themselves.

4.2 Introduction

Bacteria possess a diverse biological arsenal for killing potential competitors. Much of this arsenal involves cells producing toxic chemicals to kill other cells in the population, as in the case of allelopathy (Riley and Chavan 2007). Antibacterial toxins, known as bacteriocins, form an important part of this arsenal and are produced by almost all species of bacteria (Riley and Wertz 2002; Riley et al. 2003). Bacteriocins target specific surface receptors, and often act against strains very similar to the producing strain (Riley and Chavan 2007). This specificity enables the producing strain to target potential competitors and can be a successful mechanism to safeguard against invasion by competing strains (Brown et al. 2006). Naturally occurring microbial populations contain a variety of different bacteriocidal strains (Gordon et al. 1998; Riley and Wertz 2002) and resistance to bacteriocins, generated through mutation, is also very common in natural populations (Feldgarden and Riley 1998). Thus bacteriocins may be important in mediating both intra- and interspecific interactions such as competition for resources and may represent an important force in driving diversity through novel resistance and killing (Riley and Chavan 2007).

Altering the chemical nature of ones surroundings, via bacteriocin production, can also be considered a form of niche construction, which feeds back to affect the rest of the community (e.g. Kerr 2007). This process is ultimately frequency-dependent as the invasion of bacteriocin producers is constrained by the cost to an individual of successfully modifying the environment. In this way, a rare bacteriocin producing cell may not be able to produce enough toxin to alter the environment sufficiently for its own benefit and will therefore not compensate for the cost of bacteriocin production. Thus, bacteriocin producers will not be able to increase in frequency if they are rare (e.g. Gardner et al. 2004; Inglis et al. 2009). However, at a certain point there will be enough bacteriocin producers in the population to do

sufficient damage to competitors to compensate for production costs and they will spread. This phenomenon is known as a rarity threshold (Brown et al. 2009).

One potential way to circumvent the rarity threshold is for the trait (i.e. bacteriocin production) to be spread by more than just vertical transmission form parent to daughter cell. Bacterial genomes frequently contain vectors of horizontal gene transfer, in particular plasmids. A bacterial plasmid is a species of nonessential extrachromosomal DNA that replicates autonomously as a moderately stable component of the cell's genome (Novick 1987). They are present in all branches of the bacterial 'tree of life' and have been found in all bacterial communities studied to date (Sorensen et al. 2005). While plasmid carriage imposes a fitness cost on the host cell (Lili et al. 2007), plasmids often carry genes that are beneficial to the host, as in the case of many antibiotic resistance genes (Schumann 2001; Svara and Rankin 2011). Thus horizontal gene transfer (HGT), the non-genealogical transfer of genetic material from one organism to another (Goldenfeld and Woese 2007), is a source of new genes and functions to the recipient of the transferred genetic material (Boto 2010) and may be a potential mechanism through which to escape the constraints of initial rarity.

Bacteriocins are often carried on plasmids (e.g. Gordon et al. 2007; Heng et al. 2007). In *Escherichia coli*, for example, all bacteriocins (known as colicins) are plasmid-borne (Cascales et al. 2007). This suggests that plasmid carriage may play a role in the evolution of bacteriocins. A possible source of this advantage is that the plasmid will allow the bacteriocin to spread rapidly in the population (more rapidly than if it were carried on the chromosome and thus only spread by vertical transfer), building up sufficient toxin to compensate for production costs and thus escape the rarity threshold. Here we examine why it may be advantageous for bacteriocins to be carried by plasmids, and whether the ability to spread

horizontally as opposed to solely by vertical transmission can help overcome the invasion barrier when they are rare. We find that HGT enables bacteriocin producers to overcome the rarity threshold and that subsequently bacteriocins can allow plasmids to persist, through addiction dynamics, even after horizontal transfer is not necessary for the maintenance of the bacteriocin itself.

4.3 Method and Results

Model Structure

We start by assuming a host-associated life-cycle with a population of bacterial cells dividing into an infinite number of hosts, which we refer to herein as "patches". In our life-cycle, (1) patches are initially infected with N strains of bacteria which (2) subsequently give rise to a very large number of descendent offspring within the patch. The initial founding cells then die. Plasmids are inherited vertically during cell division, and we assume that segregation is negligible (due to its frequently negligibly low rate – Simonsen (1991)). After giving rise to a larger number of offspring, (3) transmission of the plasmid via conjugation occurs among bacteria within the patch. Transmission of a plasmid from an infected cell to an uninfected cell, upon contact between the two cells, occurs with a probability β. As plasmids exist in multiple copies within a cell, we assume that donor cells retain at least one copy of the plasmid after infecting a plasmid-free cell. After this, (4) the cells interact and bacteriocins are released. Any cells which do not contain the plasmid carrying the bacteriocin genes die. Finally, at the terminal stage of infection (5) migration occurs, with all cells competing to found new patches. Patches are cleared of all bacteria each time step and the lifecycle begins again.

To analyse our model, we start with the Price equation (Price 1970; Price 1972), which describes the change in gene frequency for a given gene, Δp and allows us to examine the effects of selection and transmission-bias separately:

$$\underbrace{\Delta p}_{\text{Change in gene frequency}} = \underbrace{\frac{1}{w}\text{Cov}[w_{ij}, p_{ij}]}_{\text{Selection}} + \underbrace{\frac{1}{w}\text{E}[w_{ij}\Delta p_{ij}]}_{\text{Transmission}}. \quad [1]$$

Here, w_{ij} is the fitness of individual i in patch j; p_{ij} is an indicator variable for carriage of a particular trait (i.e. plasmid-carried or chromosomally-carried bacteriocin production), these are random variables, and w and p are, respectively, the mean fitness and mean frequency of carriers of trait x across the whole population after transmission and selection. The covariance term refers to selection acting on a trait, while the expectation term refers to the change in gene frequency due to transmission. The change in an individual's status with respect to carriage of trait p is described by the equation Δp_{ij}. Our life-cycle assumes that transmission takes place before selection, and that there is no association between plasmid spread itself (as it takes place before selection) and fitness (Lehmann et al. 2008). We can thus express the covariance term in terms of the frequency of the gene after transmission p_{ij}^{t}. As $p_{ij}^{t} = \Delta p_{ij} + p_{ij}$, this becomes:

$$\text{Cov}[w_{ij}, p_{ij}^{t}] = \text{Cov}[w_{ij}, p_{ij}] - \text{Cov}[w_{ij}, \Delta p_{ij}].$$

We can therefore rephrase our Price equation in terms of the gene frequencies after transmission, where p_{ij}^{t} is an indicator variable of trait carriage after transmission (denoted by superscript t), as:

$$\underbrace{\Delta p}_{\text{Change in gene frequency}} = \underbrace{\frac{1}{w}\text{Cov}[w_{ij}, p_{ij}^{t}]}_{\text{Selection}} + \underbrace{\text{E}[\Delta p_{ij}]}_{\text{Transmission}}. \quad [2]$$

Both equation (1) and equation (2) show that the change in gene frequency depends on both selection (from the covariance term) and transmission-bias (from the expectation term).

Plasmid-carried bacteriocins

We assume that bacteriocin production is coded for by a gene on a plasmid. Carriage of the bacteriocin producing gene also provides immunity to the toxic effects of the bacteriocins. We denote p as the global plasmid frequency among the founding inoculum, and the frequency in late infection (after the transmission stage) is denoted p^t (where the superscript denotes that p is measured after transmission).

Transmission-Bias

For plasmid-carried genes we must calculate the transmission-bias, which is calculated by the change in plasmid carriage due only to transmission: $p^t - p$. The term p^t is the sum of those cells who carried the plasmid at the beginning of the life cycle (p) plus those plasmid-free cells ($1-p$) which were infected with the plasmid at the transmission stage by plasmid carriers from a different strain (to the plasmid-free cells): $\left(\frac{N-1}{N}\beta p\right)$. This is given by:

$$p^t = p + (1-p)\frac{N-1}{N}\beta p,$$

From this, we can calculate the transmission-bias term from equation [2], which is:

$$\mathrm{E}[\Delta p_{ij}] = \left(\frac{N-1}{N}\beta\right)p(1-p). \qquad [3]$$

Selection

We assume that a plasmid which codes for the bacteriocin will inflict a cost C on the cell, which consists of a baseline cost of carriage plus the cost of bacteriocin production. We further assume that bacteriocins kill cells which do not carry the plasmid, or are not immune to bacteriocins, and that bacteriocins thus inflict a cost v on susceptible cells. This will depend on the frequency p^t_j of bacteriocin-producers in the patch, and the cost of a susceptible cell is therefore $(1-p^t_j)v$. We can then express individual fitness after transmission as:

$$w_{ij} = 1 - Cp_{ij}{}^{t} - (1 - p_{ij}{}^{t})\, p_{j}{}^{t} v. \qquad [4]$$

Model parameters are listed in Table 1. The average fitness of all cells in the population is:

$$w = 1 - Cp^{t} - vp^{t}(1 - p^{t}) - vR^{t}\mathrm{Var}[p^{t}]. \qquad [5]$$

The term R^t refers to whole group relatedness (Pepper 2000), after transmission, with respect to the plasmid and $\mathrm{Var}[p^t]$ refers to the variance in plasmid carriage after transmission (see Appendix A equations (A1-A2) for derivation). We now use equations (2-5) to obtain the change in frequency of the plasmid over a single generation (see Appendix A equation (A3) for details).

Result 1: Bacteriocins cannot spread from rare in the absence of HGT.

In the absence of HGT (i.e. $\beta = 0$), a plasmid will be able to invade the population from rare (i.e. if p->0) if

$$0 > C \qquad [6]$$

Inequality (6) shows that, as bacteriocins inflict a net cost on the host (i.e. if $C>0$), this condition will not hold.

Result 2: If a threshold p* is reached, bacteriocins will go to fixation.

In the absence of horizontal transmission, there is a threshold frequency p^*, above which (if $p>p^*$), the plasmid is favoured, but below which (if $p<p^*$) it is disfavoured (see Figure 1). This threshold is the "rarity threshold" (Brown et al. 2009) and is given by (see Figure 2 (a)):

$$p^* = \frac{CN}{v(N-1)}.$$

This becomes clearer if we consider the case in the absence of HGT ($\beta = 0$), where the bacteriocin is stable if:

$$\frac{(N-1)v}{N} > C.$$

This shows that bacteriocin production can be stable from invasion if the gene is at fixation, but first it must overcome the "rarity threshold" (Brown et al. 2009) which initially prevents the spread of such traits. When bacteriocins are so rare that the action they take doesn't impact the competing cells, they do not obtain enough of a benefit in order to compensate the cost of bacteriocin production.

Result 3: Horizontal gene transfer favours the invasion of bacteriocins.

If there is HGT (i.e. if $\beta > 0$) the plasmid can invade from rare if the following condition holds:

$$C < \frac{N-1}{N^2}\beta\left(\frac{N^3 - v\beta^2 - vN^2(1+\beta) + Nv\beta(2+\beta)}{N^2 - \beta^2 + N\beta^2}\right). \quad [7]$$

This shows that a greater level of HGT favours the spread of the plasmid: as long as HGT is sufficiently high to be able to exceed the cost to the host cell, a plasmid carrying the bacteriocin will be able to spread through the population.

Result 4: Horizontal gene transfer promotes the stability of bacteriocins.

Once at fixation (i.e. p=1) the plasmid is then stable from invasion from non-producers if:

$$\frac{N-1}{N^2}\left(\frac{v\beta\left(N^2(\beta-3)(\beta-1) + N(3-2\beta)\beta + \beta^2\right) + N^3\left(v(\beta-1)^2 + \beta\right)}{N^2 - (N-1)N\beta + (N-1)^2\beta^2}\right) > C. \quad [8]$$

This shows that higher probabilities of HGT lead to the plasmid-carrying cells being more stable with respect to non-carrying cells (see Figure 2 (b)). In addition to this, a greater effectiveness v of the bacteriocin in killing other cells, or lower costs to a cell from being infected by a plasmid with the bacteriocin gene, the more stable the bacteriocin is to non-plasmid carrying cells.

The effects of segregation

Once the plasmid is fixed in the population, all individuals without the plasmid will be killed by the toxin and thus cannot outcompete plasmid carriers and therefore HGT is no longer required to maintain the plasmid in the population. This situation is analogous to toxin-

antitoxin plasmid addiction (Zielenkiewicz and Ceglowski 2001), however in this scenario if a cell loses the plasmid, it will be killed by bacteriocins in the environment rather than an intracellular toxin. To support this assertion, we examined the effect of segregation on the stability of bacteriocins (using the same fitness function as before), in the absence of HGT. Segregation refers to the loss of plasmids during cell replication which occurs to an individual cell with probability *s* and thus stage 3 of our lifecycle now becomes segregation and HGT is assumed not to occur. As $\beta = 0$ and we now have segregation in the model we calculate p^t as $p^t = (1-s)p$, that is, the sum of those cells who carried the plasmid at the beginning of the life cycle (*p*) and didn't lose it at the segregation state (this occurs with probability (1-*s*)). This gives $\mathrm{E}[\Delta p_{ij}] = -sp.$ The full derivation of the model is shown in Appendix B (equations (B1-B3)).

Result 5: Bacteriocins are addictive, and segregation favours plasmid stability.

We find that, in the absence of segregation the plasmid remains stable once fixed provided $v(N-1)/N > C$, as is seen in the previous models. In the presence of segregation, i.e. *s*>0, the plasmid remains stable once fixed provided:

$$v > CN/\big(N(1-s)-1\big). \qquad [9]$$

We see that plasmids can compensate for loss through segregation by increasing virulence. In this case the bacteriocins are addictive: cells which lose the plasmid also lose resistance against the bacteriocin, and are thus lost from the population (depending on how strong the effect of *v* is).

4.4 Discussion

Our model, which is based on an infinite island model, shows that bacteriocins cannot invade from rare, as rare bacteriocin-producing strains cannot kill enough competitors to compensate for the cost of bacteriocin production. While population structure aids in the invasion of toxin-producers (e.g. see Chao and Levin 1981; Wloch-Salamon et al. 2008), selection for producers remains frequency-dependent. This is particularly true in the absence of direct benefits from bacteriocin production e.g. the case of colicins, where cell lysis is required for toxin release (Cascales et al. 2007). In addition, highly diffuse bacteriocins are unlikely to generate sufficient benefits among low levels of producers. Thus the rarity threshold is likely to be an important factor for rare bacteriocin producers, which will often find it difficult to overcome and spread through the population (Brown et al. 2009).

However, this rarity threshold can be overcome if the gene in question is carried on a plasmid, and can therefore spread horizontally. Once the plasmid is established in the population, horizontal gene transfer (HGT) is no longer required to maintain it and, as long as the benefits from killing other cells, $v(N\text{-}1)/N$, are greater than the cost, C_1, of bacteriocin production, the plasmid will be stable. Previous work on temperate phage has shown that rarity threshold can be mitigated by allelopathic weapons capable of self-amplification (Brown et al. 2006). Temperate phage function as an allelopathic weapon, similar to the bacteriocins in our model. Infection with a phage causes lysis of the host cell, with the associated release of viral particles. Alternatively the virus may remain dormant in the host and be replicated vertically, spreading through the population; thus the virus is amplified in the population through both vertical and horizontal transmission (Brown et al. 2006). Our model describes a similar process whereby, in the absence of horizontal transmission, our bacteriocin trait cannot spread

from rare but can go to fixation when HGT is enabled. In our case, however, the plasmid does not necessarily kill the host, whereas in the case of the phage, even dormant phage may eventually lyse the cell. It is likely that HGT can act as an accelerant for invasion of bacteriocins in cases where direct benefits will be accrued. This may be particularly important when multiple strains are competing to invade.

Here we have described bacteriocins which are carried on a plasmid. Although this is frequently the case (e.g. Cascales et al. 2007), similar classes of bacteriocins can also be encoded on the chromosome (Michel-Briand and Baysse 2002). Our model suggests there is an initial advantage for a rare bacteriocin which is carried on a plasmid because it can overcome the rarity threshold via HGT. However, chromosomally-based bacteriocins can avoid the costs associated with plasmid carriage, which may help explain why both types exist in bacteria. During the transition phase from plasmid to chromosome, it is likely that an intermediate stage will occur where the bacterial cell will produce increased amounts of the bacteriocin complex as it is now encoded on both the plasmid and the chromosome. As such, this may impose an additional fitness cost in order to establish chromosomally-based bacteriocins. This fitness cost could perhaps be mitigated in situations where producing more bacteriocin is advantageous (i.e. conditions where there is a high density of sensitive cells in the environment or reduced potency of the toxin). An associated benefit of producing twice as much bacteriocin (or, alternatively, counteraction against over-production) could support the transition period between plasmid to chromosome. The plasmid could then be lost when less bacteriocin is needed and the cell would subsequently produce the bacteriocin without the additional cost of plasmid carriage. This may help explain why bacteriocins with similar sequence homology occur in plasmids in some bacterial species, whereas are chromosomally based in others (e.g. chromosomal S-type pyocins in *Pseudomonas aeruginosa* which have

high levels of sequence homology compared to the plasmid-borne E2 colicin of *E. coli* (Michel-Briand and Baysse 2002)).

Plasmid-based bacteriocins may face competition from other species of plasmids, with which the plasmid is incompatible (Novick 1987). This can pose a particular challenge when the competitor plasmid does not carry a costly trait (like bacteriocin production), though such plasmid competition can be ameliorated in a structured population (Mc Ginty et al. 2011). Traits which are carried on plasmids as opposed to chromosomes also risk incurring an additional cost through loss of the plasmid. Such loss, which occurs during cell division, where one of the daughter cells of the original plasmid containing cell does not inherit a copy of the plasmid, is known as segregation (Summers 1991; Møller-Jensen et al. 2000). We modeled segregation independently of transmission to look at the impact of segregation on plasmid stability (see Figure 3). Our results show that bacteriocins can act in a similar way to plasmid addiction complexes: mechanisms that ensure the stable maintenance of plasmids by killing plasmid-free segregants (Gerdes et al. 1986; Gerdes et al. 2005). The death of a plasmid-free daughter cell results from bacteriocin in the environment, as the plasmid-free cell no longer contains the mechanism to inhibit the bacteriocin's effect. This is analogous to previously described plasmid addiction complexes whereby certain gene-complexes code for both a stable internal toxin and a corresponding unstable antitoxin (Jensen et al. 1995). As long as both genes are expressed, cells carrying the complex remain healthy, but loss of the complex through segregation of the plasmid causes death, as the toxin is more durable than the antitoxin. However, in our model we make no assumptions about the stability of bacteriocins and their immunity compounds, and death is simply mediated via the absence of the plasmid-encoded immunity compound. It has been suggested that such complexes have arisen to promote plasmid persistence. Our results show that bacteriocins must sufficiently

virulent to compensate for the cost of their carriage and that this virulence is what compensates for the effects of increasing segregation (Figure 3). We can consider this scenario as plasmid addiction, i.e. a plasmid-free cell cannot survive highly virulent bacteriocin (as the genes required to inhibit the effects of the bacteriocin, are carried by the plasmid); and thus it is addicted to the plasmid.

Our model shows that, while HGT is necessary in order for bacteriocin producers to spread, once established in the population HGT is no longer required for the trait to remain stable. However, as a result of this addiction, the plasmid, i.e. the vehicle for the now redundant HGT, cannot be lost while it remains the carrier of the bacteriocin complex. In a similar manner, plasmids co-occurring with bacteriocin-carrying plasmids are prevented from segregating independently (Cooper and Heinemann 2000; Cooper et al. 2010) and also become "addicted" to the presence of the bacteriocin-carrying plasmid. This will be the case particularly for conjugative plasmids that help non-mobilisable (plasmids that are unable to independently transmit to a new host cell) bacteriocin-carrying plasmids (Ahn and Stiles 1992; Smillie et al. 2010). This can benefit conjugative plasmids which spread while remaining associated with the bacteriocin in a manner similar to genetic hitch-hiking (Maynard Smith and Haigh 1974; Barton 2000). Such a conjugative plasmid will benefit from the damage to competing cells, containing neither plasmid, caused by the bacteriocin. However, within the neighbourhood, copies of this conjugative plasmid that do not co-occur with the bacteriocin plasmid will also be killed as their host is targeted by the bacteriocin. As a result, the conjugative plasmid itself will become addicted to the bacteriocin-carrying plasmid, just as the host cell is, as the conjugative plasmid can no longer survive, in that neighbourhood, without the toxic plasmid (and its associated immunity to its bacteriocin).

Bacteriocins are an important biological phenomenon, with an estimated 99% of all bacteria thought to produce at least one bacteriocin (Klaenhammer 1988; Riley and Wertz 2002), and they play an important role in interspecific competition, virulence and social evolution (Riley and Wertz 2002; Kerr 2007). Here we show that horizontal gene transfer can help bacteriocin-producing genes to spread and established themselves in the population. Although the horizontal transfer of bacteriocins is likely to be important in explaining the maintenance and spread of these toxins, other factors such as quorum sensing (e.g. Pierson et al. 1994; van der Ploeg 2005), competition (Majeed et al. 2011) or spatial structure (e.g. Frank 1994) (which we do not explicitly consider in our model) may also be important when considering the spread of bacteriocins. Once established, the propensity to transfer horizontally is no longer essential and their own toxic effects are enough to maintain them in the population by killing non-carriers of the bacteriocin complex. It is possible that bacterial cells encounter new environments frequently enough that bacteriocins are never lost from plasmids as they may be continually challenged with new environments to invade. This helps to explain why bacteriocins are commonly carried on plasmids.

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4.5 Tables

Table 1. List of model parameters

Parameter	Definition
p	Frequency of bacteriocin plasmid carriers in the population
w	Average fitness across the population
β	Transmission probability of plasmid
s	Segregation probability of plasmid
N	Number of founder strains in a patch
C	Cost of carrying bacteriocin on plasmid
v	Death due to bacteriocin

4.6 Figure Legends

Figure 1. Horizontal gene transfer helps bacteriocins to overcome the rarity threshold.

(a) When bacteriocin producers are rare the cost of producing the bacteriocin is not compensated by competitor killing.

(b) When producers are at sufficiently high numbers they can kill competitors successfully.

(c) Horizontal transfer of the bacteriocin production trait via a plasmid helps the producers to reach sufficiently high number to make bacteriocin production a successful competition strategy.

Figure 2. Bacteriocin spread is influenced by horizontal gene transfer.

Panel (a) Threshold bacteriocin frequency (in the absence of horizontal gene transfer)

The threshold point is $p = C\,N/(N\text{-}1)v$. When the frequency of bacteriocin producers is below the line (the rarity threshold) producers cannot be maintained in the population. Parameters: C=0.1, β = 0, N = 20.

Panel (b) N = 20. The shaded areas indicate where the plasmid is stable from invasion from non-producers. This area is increased with increasing killing power (v) of the bacteriocin. Dark grey within dashed line: v =0.2. Light grey within dot-dashed line: v = 0.8.

Figure 3. The effect of increasing segregation on plasmid stability.

The area above the diagonal line shows where the plasmid remains stable ($v>CN/(N(1\text{-}s)\text{-}1)$) and the area below shows where it is unstable. C = 0.1, N = 20.

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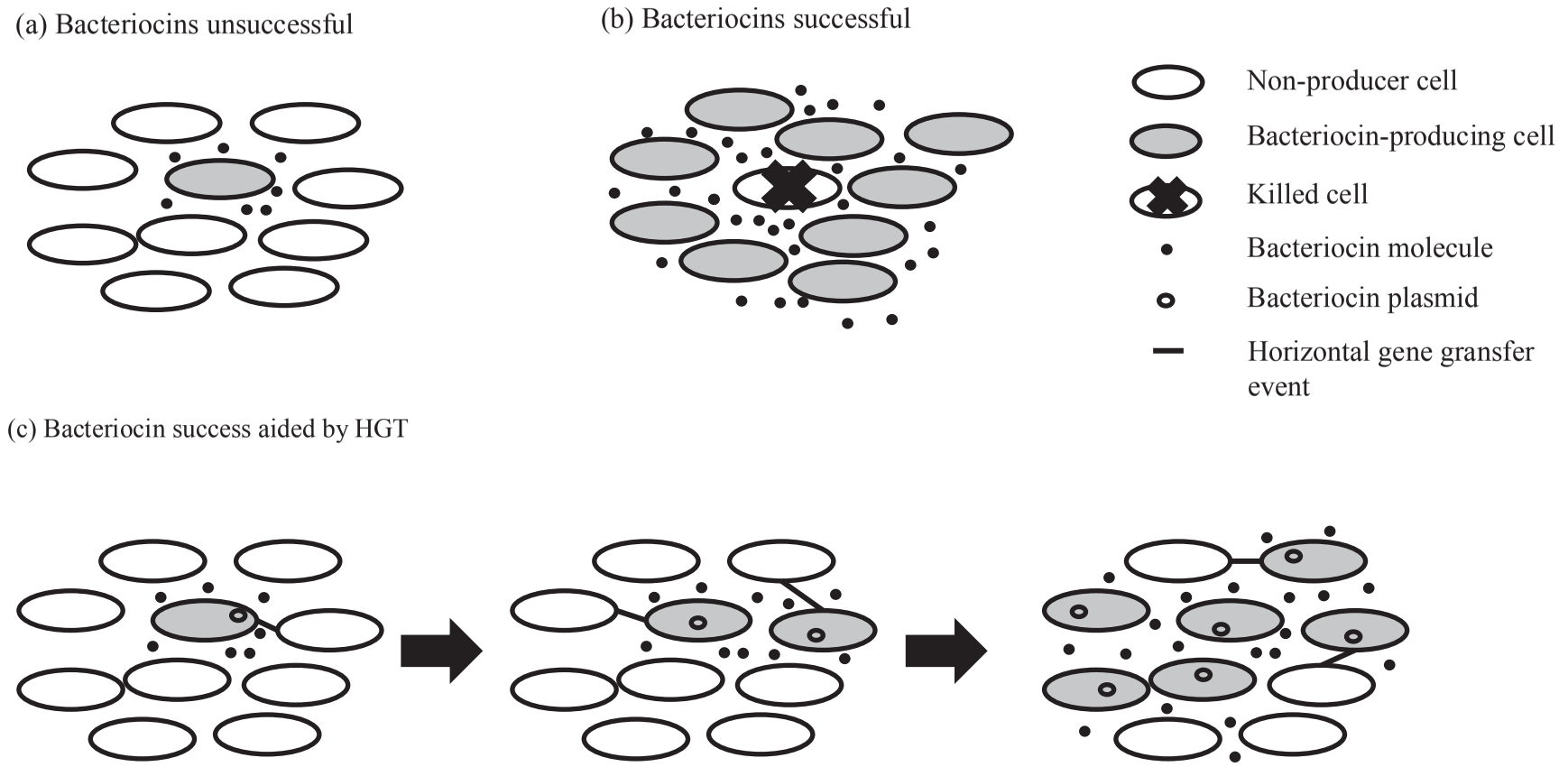
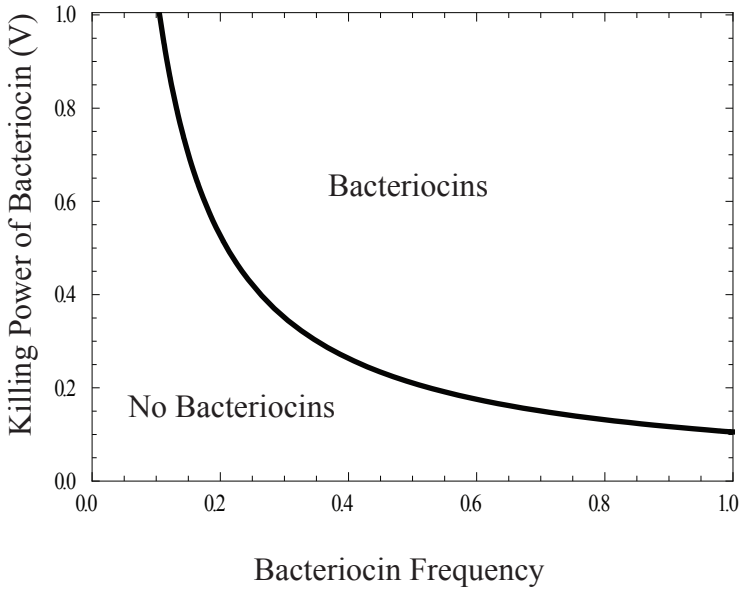

(a) Bacteriocins unsuccessful
(b) Bacteriocins successful
Non-producer cell
Bacteriocin-producing cell
Killed cell
Bacteriocin molecule
Bacteriocin plasmid
Horizontal gene gransfer event
(c) Bacteriocin success aided by HGT

Figure 1

(a)


Killing Power of Bacteriocin (V)
1.0
0.8
0.6
0.4
0.2
0.0
Bacteriocins
No Bacteriocins
0.0
0.2
0.4
0.6
0.8
1.0
Bacteriocin Frequency

(b)

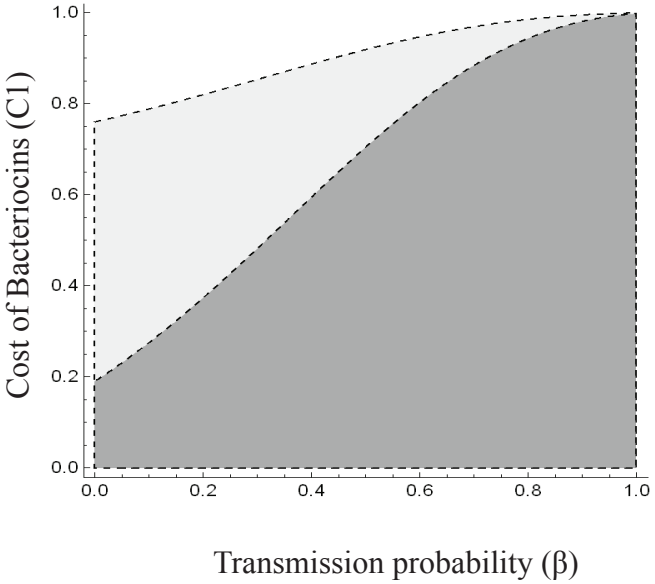

Cost of Bacteriocins (C1)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Transmission probability (β)

Figure 2

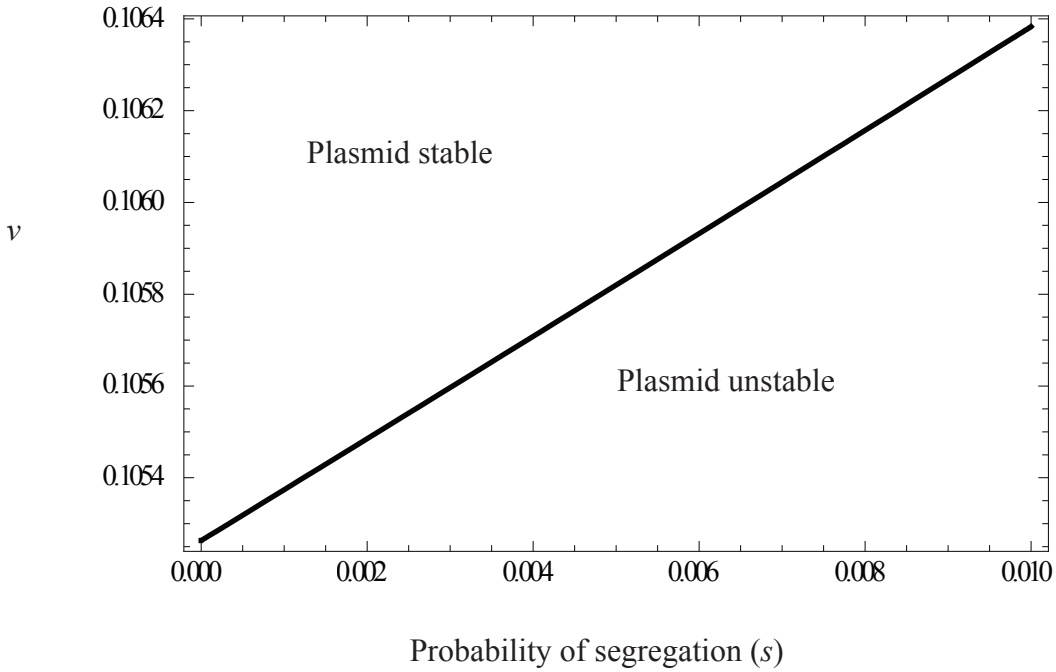

0.1064
0.1062
0.1060
0.1058
0.1056
0.1054
v
Plasmid stable
Plasmid unstable
0.000
0.002
0.004
0.006
0.008
0.010
Probability of segregation (s)

Figure 3

5 The Evolution of Conflict Resolution between plasmids and their Bacterial Hosts

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5.1 Abstract

It has recently been proposed that mobile elements may be a significant driver of cooperation in microorganisms. This may drive a potential conflict, where cooperative genes are transmitted independently of the rest of the genome, resulting in scenarios where horizontally spread cooperative genes are favoured while a chromosomal equivalent would not be. This can lead to the whole genome being exploited by surrounding non-cooperative individuals. Given that there are costs associated with mobile elements themselves, infection with a plasmid carrying a cooperative trait may lead to a significant conflict within the host genome. Here we model the mechanisms that allow the host to resolve this conflict, either by exhibiting complete resistance to the mobile element or by controlling its gene expression via a chromosomally-based suppressor. We find that the gene suppression mechanism will be more stable than full resistance, implying that suppressing the expression of costly genes within a cell is preferable to preventing the acquisition of the mobile element, for the resolution of conflict within a genome.

5.2 Introduction

Although genomes are often seen as structured entities working towards the same goal, conflict beneath the surface is in fact ubiquitous (Keller 1999; Burt and Trivers 2008). Conflict arises when individual genetic entities, be they individual genes, chromosomes or foreign DNA, such as phage or plasmids, act to maximize their own fitness at the expense of the genome as a whole. In the latter case, the genomic conflict refers to conflict between disparate genetic elements within a single cell. Plasmids are extra-chromosomal pieces of DNA which use the cell's machinery to replicate independently of the host genome (Novick 1987; Sota and Top 2008). They can be viewed as genomic parasites (Simonsen 1991), as they often code for genes which are advantageous to their own persistence and transmission, but disadvantageous to the host cell (Rankin et al. 2011b), for example, in the case of toxin anti-toxin complexes (Jensen and Gerdes 1995; Holcik and Iyer 1997; Hayes 2003). In addition, there are many costs associated with plasmid carriage (Dahlberg and Chao 2003; Wagner and Hewlett 2004; Lili et al. 2007) that lead to frequent conflict between the plasmid's replication and the survival of its host. In this sense plasmids are molecular parasites, and one may expect coevolution between the bacterial and plasmid chromosomes to facilitate a reduction in the deleterious effect of the plasmid (Bouma and Lenski 1988; Modi and Adams 1991).

Plasmids code for a diverse array of traits (Schumann 2001), but in particular it has been shown that they carry a disproportionate amount of genes involved in bacterial virulence and cooperation (Nogueira et al. 2009), suggesting a key role for plasmids in bacterial social evolution. Such traits include those which detoxify the local environment (e.g. Lee et al. 2006; Ellis et al. 2007); are involved in communication (e.g. Gonzalez and Marketon 2003; Penalver et al. 2006); and toxins (e.g. Ahmer et al. 1999). It is well established that a

cooperative gene can invade from rare in a population if $BR>C$, where B is the benefit to producing a public good, C is the cost and R is the genetic relatedness (Hamilton 1964). It has been shown that relatedness can act to promote cooperation in the production of microbial public goods (Griffin et al. 2004). Plasmids have been found to be important vectors of cooperative genes (Nogueira et al. 2009). If a gene coding for cooperation is carried on a plasmid, then such a gene will have two advantages (Smith 2001; Nogueira et al. 2009; Mc Ginty et al. 2011; Rankin et al. 2011a): the ability to spread by infecting nearby cells and an increase in relatedness between other neighbouring individuals as a result of infection (since relatedness is measured at the locus of interest, which in this case is a gene on a plasmid – Nogueira et al. 2009). Thus, the condition for a plasmid to spread from rare could be written as a modified form of Hamilton's rule, where invasion is boosted by both additional infectivity and relatedness is amplified by horizontal transfer i.e. $B(R+f)+g>C$, where f refers to the amplification of the plasmid on within-group relatedness, while g refers to the benefit to the plasmid from spreading to new cells. Both f and g would depend on factors such as the probability of transmission to an infected cell, the proportion of infected cells and the number of initial strains in a local neighbourhood. From this extended Hamilton's rule, it is evident that a plasmid carrying a gene for cooperation can spread even if $BR<C$, as it can mitigate the cost of cooperation by amplifying relatedness (through a higher f) and transmitting to other cells (through a higher g). Under this condition, there is the potential for a conflict to exist between the chromosome and a plasmid, whenever there is a net cost of a gene carried on the chromosome (i.e. if $BR<C$) but a net benefit of that same gene carried on a conjugative plasmid (i.e. if $B(R+f)+g>C$). Following the notation above, such conflict will occur if $B(R+f)+g>C>BR$, which is referred to as the "conflict zone".

A gene on a chromosome that actively suppresses expression of the cooperative gene, or prevents itself from being infected by a plasmid (which also precludes the costs associated with plasmid carriage itself), will confer an advantage over one that retains the plasmid and allows expression of the cooperative gene. As it has been shown that cooperative genes are over-represented on plasmids (Nogueira et al. 2009), it is likely that cooperative genes carried on plasmids are favoured in a way that chromosomally-carried versions are not, suggesting that conflict between plasmid-carried cooperative genes and the host chromosome may be common. As such, we would expect mechanisms from the host genome to resist the costs of cooperative plasmids which consist of both the cost of plasmid carriage and of cooperation behaviour (Bouma and Lenski 1988; Modi and Adams 1991).

A variety of mechanisms exist to defend against mobile elements (Johnson 2007) which have the potential to resolve this conflict. Here we focus on two such mechanisms: one targeting the plasmid itself and the other the cooperative gene. The first mechanism we explore is direct resistance to plasmid infection, that is, carriage of a trait that prevents conjugation or breaks down foreign DNA. This occurs in the case of restriction-modification systems (Levin 1993; Stern and Sorek 2011), or CRISPR-Cas systems (van der Oost et al. 2009; Vale and Little 2010), which act as a bacterial immune system, protecting the cell against foreign DNA. The second mechanism we examine sees the conflict resolved through a host allele which suppresses expression of the plasmid-carried gene. Such gene interactions, often involving plasmids and phages, are common in bacteria (Chernin and Mikoyan 1981; Close et al. 1985; Harr and Schlotterer 2006; Cámara et al. 2010; Gordo and Sousa 2010; Shintani et al. 2010). Here we examine the evolution of a chromosomal gene which suppresses the expression of the plasmid gene coding for the public good. We then compare the evolutionary conditions

that favour these two mechanisms to look at the evolution, and the resolution, of a conflict between plasmids and their bacterial hosts.

5.3 Model and Results

Model Structure

We use the neighbour-modulated fitness approach (Taylor and Frank 1996; Frank 1998; West and Buckling 2003; Gardner et al. 2004) to model a large population of host-associated bacteria which are subdivided into an infinite number of hosts which we refer to herein as "patches". Our life cycle consists of five stages. (1) Founding: Each patch is initially infected with an inoculum of N founder strains randomly sampled from an infinite pool of potential founder strains. We assume that, at this stage, all cells are capable of reproducing. (2) Proliferation: Founder cells divide and grow to a large number within the patch, meaning that (in the absence of transmission) the whole-group relatedness is $R=1/N$. Founder cells die after reproduction. Bacteria may be plasmid-carriers or plasmid-free and plasmids are inherited vertically during cell division. As the rate of segregation is generally on the order of 10^{-6} or lower (Simonsen 1991), we assume that segregation is negligible, and do not include it in our models. (3) Horizontal gene transfer (HGT): We model transmission using the probability β, which is the probability that, given that an uninfected cell meets an infected cell, the plasmid will be transmitted. For simplicity we assume that only uninfected cells can acquire a plasmid during this stage and conjugation therefore requires an uninfected cell to make contact with an infected cell, before conjugation can occur. (4) Fitness Interaction: Offspring survival is determined by results of interaction between cells based on the fitness function described below. (5) Dispersal: Any associations between the two loci are disrupted by recombination and all descendant bacterial cells compete globally to found new patches. Unsuccessful

bacteria (i.e. those that fail to infect a new patch) die. Every time step patches are cleared of all bacteria (either immune clearance or patch extinction/host death), and the lifecycle begins again.

We begin with the Price equation (Price 1970; Price 1972), which describes the change in gene frequency for a given gene, and allows us to partition the effects of both selection and and transmission on the change in gene frequency:

$$\underbrace{\Delta p_x}_{\text{Change in gene frequency}} = \underbrace{\frac{1}{w}\text{Cov}[w_{ij}, p_{xij}]}_{\text{Selection}} + \underbrace{\frac{1}{w}\text{E}[w_{ij}, \Delta p_{xij}]}_{\text{Transmission}} \quad [1]$$

where w_{ij} is the fitness of individual i in patch j, p_{xij} is an indicator variable describing whether individual i in patch j carries trait x (i.e. the plasmid or the resistance trait, taking a value of one if it has the given gene and zero if it doesn't), w_{ij} and p_{xij} are random variables and w and p_x are, respectively, the mean fitness and mean frequency of carriers of trait x across the whole population after transmission and selection. In equation (1) (and all following analyses), both the covariance term and expectation term are taken over all individuals (i) in all patches (j).

Model 1 – Cooperative plasmids and chromosomal resistance

We start by investigating the condition for a plasmid which carries a gene for cooperation to invade. We denote p_1 as the global plasmid frequency among the founding innoculum, and the

frequency after the transmission stage is denoted p_1^t (where the superscript denotes that p_1 is measured after transmission). The plasmid comes at a cost C_1 to the host cell, and confers a benefit B on other cells in the local environment. C_1 consists of a baseline cost of carriage (v) in addition to a cost for expression of social behavior (C) (so that $C_1=C+v$). We assume that some cells carry a gene which confers resistance to a plasmid, and thus cannot be infected by a plasmid. The global frequency of such resistant cells is denoted by p_2. Such resistance incurs a cost C_2 to the resistant individual and can be seen as a form of bacterial immune system, which would protect the cell against foreign DNA, such as plasmids. For example, this could come either in the form of a CRISPR-Cas system, where an infecting plasmid would be removed from the bacterial host (Sorek et al. 2008; van der Oost et al. 2009; Vale and Little 2010) or a restriction modification system whereby foreign DNA is degraded by a restriction endonuclease (Wilson and Murray 1991; Stern and Sorek 2011). As we assume full effectiveness of the resistance gene, individuals who carry p_2 cannot carry p_1 and vice versa. Individual fitness after transmission and interaction is therefore:

$$w_{ij} = 1 - C_1 p_{1ij}{}^{t}(1 - p_{2ij}{}^{t}) - C_2 p_{2ij}{}^{t}(1 - p_{1ij}{}^{t}) + B p_{1j}{}^{t}. \qquad [2]$$

The terms used to calculate fitness are listed in Table 1. The average frequency of the plasmid after transmission (p_1^t), is the sum of those cells who carried the plasmid at the beginning of the life cycle (p_1) plus those plasmid-free cells which were infected with the plasmid at the transmission stage (as they also do not carry the resistance trait) such that

$p_1^{\,t} = p_1 + (1 - p_1 - p_2)\frac{N-1}{N}\beta p_1$. The frequency of resistance gene after transmission is

denoted as $p_2^{\,t}$ (where $p_2^{\,t} = p_2$), since resistant individuals are not affected by plasmid transmission.

Rearranging equation (1), as described in Appendix A, and applying the fitness term above gives the change, over one generation, in plasmid and resistance frequency from one generation to the next in p_x, in terms of $p_x^{\,t}$:

$$\Delta p_1 = p_1^{\,t}(1-p_1^{\,t})\left(\frac{BR-C_1}{1+Bp_1^{\,t}-C_1p_1^{\,t}-C_2p_2^{\,t}}\right)+p_1^{\,t}p_2^{\,t}\left(\frac{C_2}{1+Bp_1^{\,t}-C_1p_1^{\,t}-C_2p_2^{\,t}}\right)+\Delta p_1^{\,t} \qquad [3a]$$

$$\Delta p_2 = \frac{1}{w}\left(p_1^{\,t}p_2^{\,t}\left(BQ_{12}+C_1\right)-C_2p_2^{\,t}(1-p_2^{\,t})\right) \qquad [3b]$$

Where $\Delta p_1^{\,t} = p_1^{\,t} - p_1 = (1-p_1-p_2)\frac{N-1}{N}\beta p_1$; R refers to whole group relatedness with respect to the plasmid, measured after transmission ($R = \mathrm{Cov}[p_{1j}^{\,t}\,p_{1j}^{\,t}]/\,p_1^{\,t}(1-p_1^{\,t})$); and Q_{12} refers to the within-patch association between plasmid carriers and resistance cells ($Q_{12} = \mathrm{Cov}[p_{1j}^{\,t}\,p_{2j}^{\,t}]/\,p_1^{\,t}p_2^{\,t}$), also measured after transmission (see Appendix A for details).

Result 1: Plasmid cooperation leads to conflict with the chromosome.

From equation (2a), we find that, in the absence of transmission, the cooperative plasmid may spread provided $B\frac{1}{N} > C_1$, when both the plasmid and the resistance gene are rare. This is essentially a version of Hamilton's rule $BR > C$ where R=1/N refers to "whole group relatedness" (Pepper 2000). If the plasmid can transmit horizontally (i.e. if β>0), a cooperative plasmid will spread from rare (when the resistance gene is also rare) under less stringent conditions (where $C_1 = C + v$):

$$B\left(\frac{1}{N} + \beta\frac{N-1}{N}\frac{1}{N}\right) + \beta\frac{N-1}{N+\beta(N-1)} > C + v\,. \qquad [4]$$

It is clear that, if $C+v>B/N$, but inequality (4) still holds, there will be a conflict between a cooperative gene carried on a plasmid, with the bacterial chromosome. Figure 1 illustrates this conflict between the chromosome and the plasmid, where the cooperative behaviour is favoured by spreading horizontally in scenarios where the trait would not be favoured if it were chromosomally based. This conflict is driven by the two reasons why cooperative genes are favoured on plasmids (Rankin et al. 2011a): the first is due to infectivity, where the plasmid simply spreads to previously uninfected hosts (which, when the plasmid is rare, is given by the term $b(N-1)/(N+b(N-1))$ in inequality (4)), while the other is due to an amplification of local genetic similarity in a patch (which, when the plasmid is rare, is given by the term $\beta(N-1)/N^2$ in equality (4)).

Result 2: HGT does not affect invasion of resistance.

The condition for the resistance gene to invade a population where the cooperative gene is at fixation when there is no HGT (i.e. when β=0) is $C_1 - B/N < C_2$. This shows that resistance evolves independently of the degree of HGT (β>0) as the resistance mechanism blocks the transmission of the plasmid into uninfected cells, and thus removes the advantage of horizontal transfer. However, the cooperative gene will only invade if it is on a plasmid if β>0 when C_1>B/N (from inequality (4)).

Result 3: Full resistance frequently leads to cyclical dynamics.

Figure 2A shows the dynamics of the plasmid versus the resistance gene. We use a parameter set that allows coexistence and we see that this coexistence displays cyclical dynamics suggesting that this mechanism of conflict resolution will frequently be unstable and will result in cycles between resistant cells, plasmid-infected cells and cells which carry neither trait.

Model 2 – Chromosomal suppression of plasmid gene expression

In our previous model, resistance against the plasmid acted by preventing infection of a cell with the plasmid. Here we focus on an alternative method, namely suppression of the plasmid-carried cooperative gene, which targets only the social trait carried on the plasmid not the plasmid itself. We assume that a suppressor carried on the host chromosome can act to decrease/fully suppress expression of specific plasmid genes (it may, of course, also function to enhance plasmid gene expression but we focus here on suppression in order to decrease the conflict between chromosome and plasmid). We assume that the plasmid can still transfer to

plasmid-free cells by conjugation, regardless of whether the cell carries the suppressor allele. Thus, cells carrying the suppressor can be infected with the plasmid but their expression of the plasmid-borne cooperative trait is reduced (see below). The global frequency of plasmid carriers is denoted by p_1, while p_2 now refers to the global frequency of cells carrying the suppressor trait. The fitness function for this model is:

$$w_{ij} = 1 + Bp_{1j}{}^{t}\left(1 - hp_{2j}{}^{t}\right) - Cp_{1ij}{}^{t}\left(1 - hCp_{2ij}{}^{t}\right) - C_2 p_{2ij}{}^{t} - vp_{1ij}{}^{t}. \qquad [5]$$

Here C refers solely to the cost of expressing the cooperative gene and the cost of plasmid-carriage is denoted by v (this is not affected by the suppressor gene). The suppressor affects expression of the cooperative trait in cells which carry both the suppressor and the plasmid. The suppressor's effect on the costs and benefits associated with the expression of the cooperative trait (C and B) is controlled by parameter h (where h=0 results in full expression of the cooperative trait and h=1 in full inhibition of the cooperative trait by the suppressor). $p_1{}^{t}$ is the sum of those cells who initially carried the plasmid plus those plasmid-free cells which were infected with the plasmid at the transmission stage (regardless of whether or not they carried the suppressor gene) such that $p_1{}^{t} = p_1 + (1 - p_1)\frac{N-1}{N}\beta p_1$. As in the previous model, p2 is not transmitted horizontally and therefore $p_2{}^{t} = p_2$. As before, the terms used to calculate fitness are listed in Table 1.

As before, we can use the Price Equation (equation (1)) to describe the changes in the frequency of the suppressor and the plasmid from one generation to the next as (see Appendix B for details):

$$\Delta p_1 = \frac{p_1^{\,t}(1-p_1^{\,t})\left(BR - C - v - p_2^{\,t}(BR-C)h\right)}{1 + Bp_1^{\,t}\left(1-hp_2^{\,t}\right) - Cp_1^{\,t}\left(1-hp_2^{\,t}\right) - C_2 p_2^{\,t} - vp_1^{\,t}} + \Delta p_1^{\,t} \qquad [6a]$$

$$\Delta p_2 = \frac{\left(p_1^{\,t} p_2^{\,t}\left(B(Q_{12} - hp_2^{\,t}Q_{23}) + hC(1-p_2^{\,t})\right) - C_2 p_2^{\,t}(1-p_2^{\,t})\right)}{1 + Bp_1^{\,t}\left(1-hp_{2j}^{\,t}\right) - Cp_1^{\,t}\left(1-hp_2^{\,t}\right) - C_2 p_2^{\,t} - vp_1^{\,t}}, \qquad [6b]$$

where $\Delta p_1^{\,t} = (1-p_1)\frac{N-1}{N}\beta p_1$; R again refers to whole group relatedness with respect to the plasmid; Q_{12} refers to the association between the plasmid carriers and suppressor carriers; and Q_{23} refers to the association between suppressor carriers and cells which carry both the suppressor and the plasmid (where individuals who carry both alleles are denoted by the subscript "3") - see Appendix B for details.

Result 4: Different mechanisms of conflict resolution show similar invasion criteria.

As before, the plasmid can spread in the absence of both transmission and the conflict resolution mechanism (in this case the suppressor gene) if $BR > C + v$ (where whole-group relatedness is given by $R = \frac{1}{N}$) . As we assume a cost to the suppressor gene, it cannot spread to fixation in the population in the absence of the plasmid (as we assume that C_2>0). The suppressor gene can spread from rare when the plasmid is at fixation if

$$hC > C_2 + hB\frac{1}{N},$$

which requires that $1+B>C+v$. This condition is similar to that of the resistance gene invading a population fixed for plasmids ($C_1 > (1/N)B + C_2$).

Result 5: Horizontal gene transfer remains advantageous for invasion when the suppressor is at fixation

When the suppressor gene is at fixation, the cooperative plasmid cannot invade from rare in the absence of HGT. However, in contrast to model 1, in the presence of HGT, the plasmid can invade the suppressor at fixation (i.e. if $p_2 \rightarrow 1$ and $h=1$), provided $(N-1/N)\beta > x/(C_2 + x - 1)$ and $C_2<1$. While the suppressor controls the expression of the cooperative trait (such that the host cell is no longer at the mercy of the plasmid's social behaviour), it does not impact on the transfer of the plasmid.

Result 6: Suppression of cooperative genes is a more stable form of defense against plasmids than full resistance

Figure 2B shows that the plasmid and the suppressor gene can lead to stable coexistence. However, we do not observe cycling between the suppressor gene and the plasmid, as we did in model 1 (see Result 3). The suppressor mechanism allows for plasmid carriage, and simply inhibits expression of the genes carried on the plasmid, while "chromosomal resistance" (i.e. prevention of carriage of the cooperative gene altogether) does not allow for a plasmid to be carried in a cell carrying the resistance gene. Thus, chromosomal resistance can be seen as analogous to host-parasite or predator-prey interactions, where the resistance gene has a

strong impact on the fitness of the plasmid, and thus leads to intransitive dynamics. Thus full resistance (model 1) leads to cycles of plasmid-carriers, resistance-carriers and empty cells (model 1), while suppression (model 2) allows for stable coexistence with both the plasmid and the suppression gene being able to go to fixation.

5.4 Discussion

Our models investigate the coevolution between plasmids and the host chromosome, specifically when plasmids carry genes coding for a cooperative trait. Plasmids can be seen as selfish genetic elements, which replicate independently of the host chromosome, and our study has examined the mechanisms by which bacterial chromosomes can mitigate the costs of carrying such selfish mobile elements. In our model, the mitigation of costs occurs either by resisting plasmid infection or by regulating certain aspects of plasmid gene expression. In the case of chromosomal resistance, we find that the coevolutionary dynamics of plasmid carriage and chromosomal resistance to a plasmid frequently results in non-transitive cycling between plasmids, resistance genes and wild-type cells (i.e. cells without either the plasmid or the resistance gene – Figure 2). These non-transitive dynamics reflect rock-paper-scissors dynamics (Sinervo and Lively 1996; Kerr et al. 2002), a common phenomenon in coevolutionary interactions. We do not observe cycling in our model incorporating a suppressor mechanism (due to the fact that a cell can simultaneously carry both the plasmid and the chromosomal suppressor gene leading to stable coexistence), suggesting that resistance is likely to be less stable than suppression of plasmid genes as a mechanism to resolve the conflict between a plasmid and the bacterial chromosome.

Genomic conflict is prevalent at all levels of biological organization (e.g. Keller 1999) and here we examine the existence of a conflict between a host chromosome and its plasmid with respect to cooperative traits, first highlighting the region where conflict will occur (Figure 1). Plasmids have been previously suggested as a strategy for a gene to impose a particular phenotype on cells (Smith 2001): the ability to force a host to express given genes can result in a cell performing certain behaviours (e.g. cooperation) in a way that is suboptimal for the chromosome, but beneficial for the plasmid carrying such a gene (as seen here, when plasmid-carried cooperation is favoured even though $0<BR<C$). Horizontal gene transfer can thus help to promote the spread of genes that would not otherwise be favoured in the absence of horizontal transmission (Smith 2001; Nogueira et al. 2009; Mc Ginty et al. 2011). Horizontal gene transfer itself can therefore drive conflict between the chromosome and the plasmid (Figure 1).

Full resistance may appear to be the most effective way to deal with such genes because it completely removes their advantage of horizontal spread whilst also negating the cost of plasmid carriage for the host. However stable coexistence between the plasmid and the resistance gene is not possible and resistance frequently results in non-transitive cycling. Most forms of resistance in host-parasite systems involve costs to the host (Sheldon and Verhulst 1996), which determine both the resulting co-evolutionary dynamics and the extent to which there is variance in the effects of different resistance genes (Antonovics and Thrall 1994). Although measurements of costs of resistance are limited (Lennon et al. 2007), in the case of CRISPRs, it is likely that the costs of resistance will be associated with the length of, or the number of, CRISPRs in the genome (Vale and Little 2010).

Our second model involves a host-expressed suppressor, which reduces (or entirely prevents) the expression of the plasmid-carried gene (here, a social trait). In contrast to the full resistance model, gene suppression does not exhibit cycling, primarily because plasmids maintain the ability to propagate. It is possible that gene regulation mechanisms may inflict lower costs than complete resistance: for example a mechanism such as methylation is likely to be relatively low-cost as it is a common feature of cell development (Jaenisch and Bird 2003). However, gene regulation is not without its drawbacks. Conflict arises both from the carriage of the plasmid but also from the expression of its genes. Therefore the advantage of gene regulation as a mechanism to counter the costs of plasmid infection, over full resistance, will depend upon the costs of plasmid carriage and the net cost of expressing genes carried on a plasmid. While we have not looked at competition between resistance and suppression directly, we would expect that resistance will be more likely to evolve if there is a higher cost to plasmid carriage (i.e. if v is high), as opposed to suppression, which will evolve if there is a higher net cost of expressing genes on the plasmid (i.e. if C is high, and $C>BR$, even if the costs v from plasmid carriage are high).

Our results highlight the causes and consequences of coevolution between plasmids and their bacterial hosts. Bacteria have previously been shown to adapt to the presence of plasmids (Bouma and Lenski 1988; Modi and Adams 1991), and plasmids themselves may also adapt to the host (Modi and Adams 1991). In our model, the host chromosome evolves a given mechanism to mitigate the cost of plasmid carriage, whether that is resistance (in model 1 – the "resistance" model) or suppression of plasmid gene expression (in model 2 – the "suppression" model). In the case of the resistance mechanism, it is possible that the plasmid may evolve to counter the influence of resistance, a phenomenon that has been observed to occur in CRISPR systems (Semenova et al. 2011). In contrast to an outright resistance

mechanism, a gene regulation mechanism may be more stable because it still allows the plasmid to propagate, and thus has the potential to reduce the selective pressure for plasmid counter-adaptations. It is thus possible that coevolution between the plasmid and the host itself may mitigate the cost caused by carriage of the plasmid by reducing the cost of plasmid carriage without the need to develop costly systems such as CRISPRs (Bouma and Lenski 1988; Modi and Adams 1991).

There are other ways in which the costs of plasmid carried cooperation can be reduced, which we have not considered in our model. For example, the reduction of the number of plasmids carried by a host (i.e. the copy number of the plasmid – Iordanescu and Bargonetti 1989) or an increase in the rate of plasmid segregation (e.g. Modi and Adams 1991) could reduce the costs imposed by a plasmid on the host cell. Large plasmids are suggested to be the origin of bacterial secondary chromosomes (Smillie et al. 2010), as has been shown in *Rhizobiaceae* (Slater et al. 2009). Conversion of a plasmid to a secondary chromosome may be viewed as a potential mechanism of conflict resolution where the ability of a plasmid to transfer to other hosts would be reduced, for example by their own fertility inhibition systems (Dionisio et al. 2002; Haft et al. 2009), which would then help to align the interests of the plasmid and the host chromosome (Dahlberg and Chao 2003).

Our model has shown that, when a plasmid carries genes involved in cooperation, there is the potential for a conflict between the plasmid and the host chromosome. These results suggest that the mechanisms that mitigate the costs of plasmid gene expression will generally be more stable, than full resistance mechanisms. Our model bears similarities to other conflict resolving mechanisms such as policing in social insects (e.g. Foster and Ratnieks 2000),

where “suppression” would be seen as analogous to the “policing” which act to the detriment of the colony. In policing, reproduction by workers is repressed by policing individuals, for the good of the colony (e.g. Foster and Ratnieks 2000). The wider implication is that “policing”, or suppression of genes already within a cell, is a more stable option for a host cell to resolve a genetic conflict than actively inhibiting the spread of the mobile element. However, as a gene regulation mechanism merely targets gene products and not the plasmid itself, only full resistance mechanisms can eliminate conflict, however briefly, between plasmids, and their bacterial hosts.

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5.5 Tables

Table 1. List of terms used to generate fitness function *w*. All terms refer to both resistance and suppressor models unless otherwise stated.

Term	Definition
w_{ij}	Fitness of an individual *i* in patch *j*. This is a random variable.
w	Global mean fitness across all individuals in all patches in the population such that $w = \mathrm{E}[w_{ij}] = \sum_{ij} w_{ij}/(Nn)$.
p_{xij}	An indicator variable that takes the value 1 if the individual carries allele *x* and 0 otherwise. This is a random variable. A superscript *t* indicates when this is measured after transmission.
p_x	Global mean frequency of allele *x* across all individuals in all patches in the population such that $p_x = \mathrm{E}[p_{xij}] = \lim_{n\to\infty}\left[\sum_{ij} p_{xij}/(Nn)\right]$. A superscript *t* indicates when this is measured after transmission.
Plasmid carriers (p_1)	Carries a trait which provides benefit *B* to all individuals within the same deme (*j*) coming at a cost *C* to the cooperative individual (*i*)
Resistance/suppressor carriers (p_2)	Resistance model: Carries resistance to infection by plasmids Suppressor model: Carries an inhibitor which prevents expression of the plasmids cooperative behaviour
N	Number of founder strains on each patch

Table 1. continued

Term	Definition
n	Number of patches in the population
β	Transmission probability of the plasmid
C	Cost of cooperative behaviour to the cooperative individual (i)
v	Baseline cost of plasmid carriage
C_1	Cost of cooperative behaviour to the cooperative individual (i) plus baseline cost of plasmid carriage i.e. $C_1=C+v$
C_2	Resistance model: Cost of expressing resistance Suppressor model: Cost of expressing supressor
h	Suppressor model: effect of suppressor on cooperative gene expression and its associated costs and benefits i.e. h=1 gives full suppression of the cooperative trait and h=0 gives no suppression (i.e. full expression) of the cooperative gene
B	Benefit of cooperative behaviour. Shared by all individuals within the same deme (j)

5.6 Figure Legends

Figure 1. Conflict between the host chromosome and the plasmid with respect to cooperative traits

The areas favouring each scenario are denoted. The area where it is only in the plasmids interest for the cell to cooperate, but not for the chromosome, can be seen as the "conflict zone". Chromosomal cooperation is favoured when $B\frac{1}{N} > C_1$, whereas plasmid-based cooperation is favoured when $B\left(\frac{1}{N} + \beta\frac{N-1}{N}\frac{1}{N}\right) + \beta\frac{N-1}{N+\beta(N-1)} > C_1$ allowing plasmid-carried cooperation to persist in areas where $B\frac{1}{N} < C_1$. Parameters: β = 0.05, C_1= 0.09, B = 0.3.

Figure 2. Dynamics of two mechanisms of defence against plasmids.

Parameters: β = 0.2, B = 0.4, N = 100. For parameter values where there is coexistence between the resistance mechanism and the plasmid full resistance results in cyclical dynamics whereas in contrast both traits can go to fixation for the suppressor model. Panel (a) model (1) resistance to plasmids, C_1 = 0.18, C_2 = 0.05. Panel (b) model (2) suppression of cooperative behaviour, v = 0.05, C_1 = 0.13, C_2 = 0.05, h=1.

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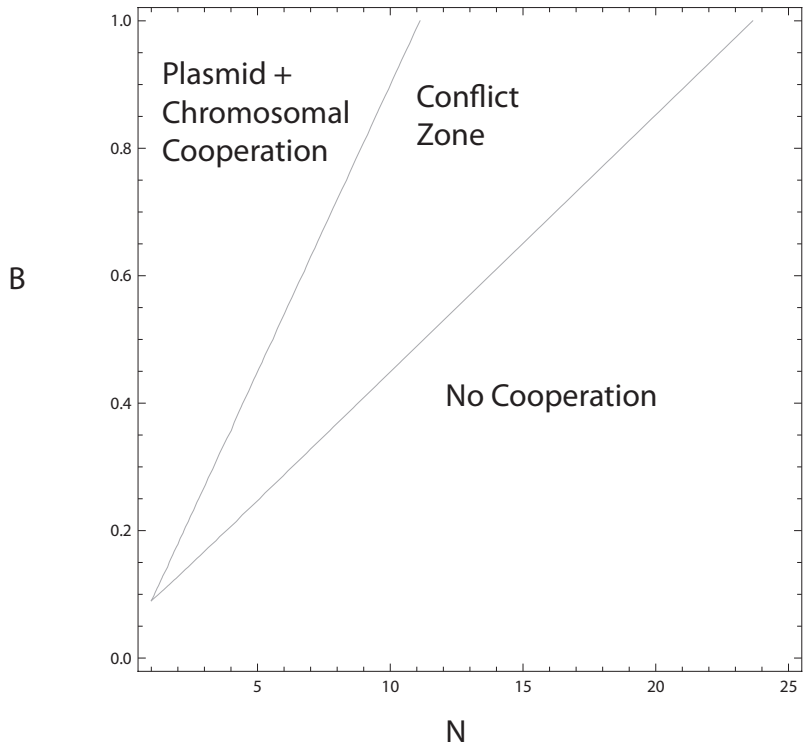
Plasmid +
Chromosomal
Cooperation
Conflict
Zone
No Cooperation
B
N
1.0
0.8
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10
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25

Figure 1

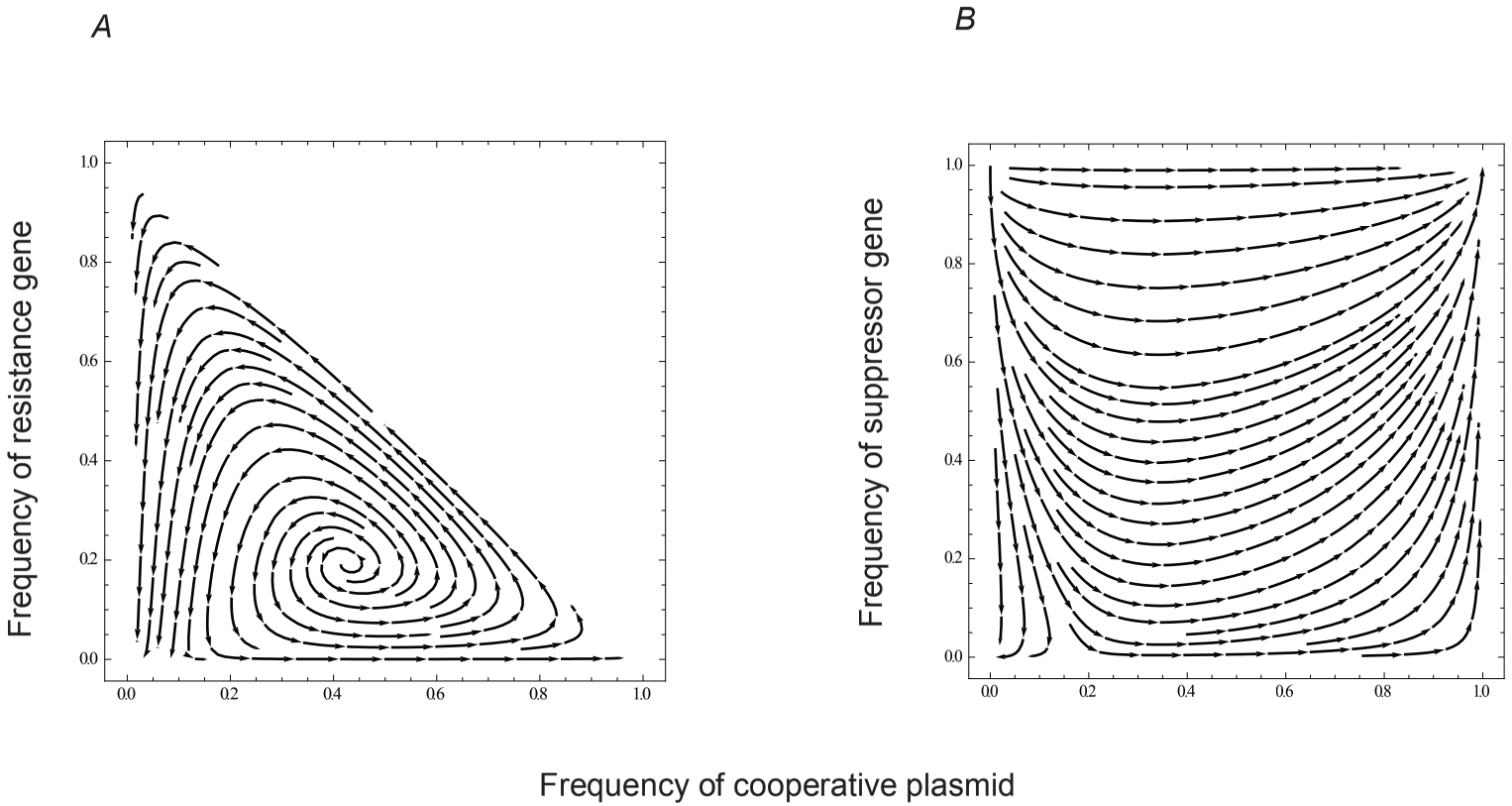
A
B
Frequency of resistance gene
Frequency of suppressor gene
Frequency of cooperative plasmid
0.0
0.2
0.4
0.6
0.8
1.0

Figure 2

6 Horizontal Gene Transfer and the Evolution of Antibiotic Resistance

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6.1 Abstract

Antibiotic resistance is one of the traits most commonly spread by horizontal transmission. Plasmids are important vectors of antibiotic resistance genes. Here we investigate the spread of plasmids carrying antibiotic resistance genes in a metapopulation using a model based upon their within-host dynamics. We examine the spread of resistance plasmids in competition with a chromosomal variant in addition to incorporating the effects of plasmid competition by modeling the dynamics between two incompatible plasmids. We show that generally, plasmid-encoded resistance is more likely than chromosomally-encoded resistance. Our results further show that plasmid resistance will dominate a metapopulation when this form of resistance is as likely to encounter chromosomal resistance as it is susceptible pathogens. Plasmid competition leads to an overall reduction in resistance in the absence of antibiotic treatment but, upon addition of antibiotics, favours chromosomally-encoded resistance or carriers of the resistance gene on the chromosome as well as on a plasmid. Plasmid-carried resistance has previously been studied within hosts, but our results show that features of structured populations are important in determining the success of plasmid-spread resistance and the effects of competition with chromosomal variants and other plasmids thereon.

6.2 Introduction

Antibiotic resistance in pathogenic bacteria is one of the foremost challenges to public health in recent times. Resistance to antibiotics has arisen against a variety of drugs (Alekshun and Levy 2007) and can take place through a variety of mechanisms including efflux pumps, mutation to target proteins and antibiotic inactivation (Allen et al. 2010). Microbes are able to rapidly evolve resistance to antibiotics through these different mechanisms, and thus controlling antibiotic resistance is a major challenge. While resistance is frequently a chromosomally-based trait (Alekshun and Levy 2007), horizontal gene transfer (HGT) plays a large role in the spread of antibiotic resistance genes. HGT is recognized as a means of bacterial innovation whereby useful traits are spread within and among bacterial species (Ochman et al. 2000). In such a way resistance traits have the potential to spread in the absence of selection.

HGT occurs by three mechanisms: transformation (uptake of naked DNA from the environment), transduction (via phage) and conjugation (via plasmids); of these three mechanisms transduction and conjugation frequently carry resistance-associated traits (Eberhard 1990; Bennett 2008). Plasmids are extrachromosomal pieces of DNA which are capable of replicating independently of the host genome (Novick 1987; Sota and Top 2008). They often spread multiple genes and it has been found that multiple antibiotic resistance genes are frequently arranged in clusters on plasmids (Barlow 2009), thus allowing the rapid dissemination of a variety of resistance traits together. Genes for resistance can therefore be present simultaneously on chromosomes and on plasmids.

While it has long been known that plasmids play an important role in the transfer of antibiotic resistance genes (Datta and Kontomic 1965), it is still not known which selective pressures favour antibiotic resistance plasmids when a chromosomal variant is available. Various factors relating to antibiotic treatment such as dosage of the antibiotics applied and the interval between treatments have important effects on the success of resistance plasmids (Svara and Rankin 2011). It has also been proposed that any useful plasmid-carried trait will be eventually incorporated into the bacterial chromosome (Bergstrom et al 2000). As plasmids themselves impose a cost on their host (Lili et al. 2007) it remains to be elucidated how such resistance persists in the presence of a chromosomal variant except by virtue of HGT.

Here we aim to examine the spread of antibiotic resistance plasmids in a metapopulation using a model based upon their within-host dynamics. The evolution of plasmid-carried resistance has previously been studied within hosts (Svara and Rankin 2011), but it remains important to examine this phenomenon in a structured population reflecting how infectious diseases themselves are typically studied. We further extend our analyses to include what is likely to be a common feature: competition with incompatible plasmids. We examine the success of plasmid-transmitted resistance in competition with a chromosomal variant in both of these scenarios with a view to determining why plasmid-spread resistance is so successful.

6.3 Model and Results

Resistance plasmids: Within-patch population dynamics

Our within-host model focuses on the dynamics of a wild-type pathogen in competition with cells of the same strain which carry resistance to antibiotics. We first describe the dynamics in the absence of antibiotics. In the absence of antibiotics we model the wild-type pathogenic strain (referred to as *F*, the density of which is denoted n_{Fo}, where the subscript *o* indicates the absence of antibiotics); the wild-type is not resistant to antibiotics. We also model a strain carrying an antibiotic-resistance gene on a plasmid (*P*, with a density of n_{Po}); a strain carrying the same antibiotic-resistance gene on its chromosome (*C*, density n_{Co}); and a strain that carries the antibiotic-resistance on both its chromosome and a plasmid (*CP*, density n_{CPo}). We assume logistic growth, with a growth rate of *r* and a density-dependent death rate μ, where μ is the growth rate, *r*, divided by the carrying capacity, *k*. The total size of the population is denoted by *N*. Plasmid carriage entails a cost *v* and resistance to antibiotics entails cost *c*. Model parameters are listed in full in Table 1. The plasmid can infect both wild-type (*F*) and chromosomally-resistant cells (*C*) at rate β_1. We describe the population as follows:

$$\begin{aligned} dn_{Fo}/dt &= n_{Fo}\left(r - \mu N - \beta_1 n_{Po} - \beta_1 n_{CPo}\right) \\ dn_{Po}/dt &= n_{Po}\left(r - c - v - \mu N + \beta_1 n_{Fo}\right) + \beta_1 n_{CPo} n_{Fo} \\ dn_{Co}/dt &= n_{Co}\left(r - c - \mu N - \beta_1 n_{Po} - \beta_1 n_{CPo}\right) \\ dn_{CPo}/dt &= n_{CPo}\left(r - c - v - \mu N + \beta_1 n_{Co}\right) + \beta_1 n_{Po} n_{Co}. \end{aligned} \qquad [1]$$

We can analyse this model to determine both the equilibria and the invasion success $\left(\frac{\partial(dnx/dt)}{\partial nx}\right)$ of the individual strains (where $x = \{F, P, C, CP\}$) into these equilibria. The

results are listed in Table 2. We find that, as expected, the chromosomal resistance allele is outcompeted by the wild-type in the absence of antibiotics due to the cost associated with resistance. In addition, the wild-type is displaced by the resistance plasmid-carrying strain which has the advantage of horizontal gene transfer and can invade when its basic reproductive number, R_0, is greater than 1, i.e. when $c+v<k\beta_1$. Similarly, under these conditions the chromosomally resistant strain (*C*) is also invaded by plasmid carrying individuals (*CP*). In fact, resistance carried via the chromosome can only be maintained in the population in the form of cells carrying both chromosomal and plasmid resistance.

In the presence of antibiotics we assume the wild-type strain cannot survive leaving only a resistance plasmid-carrying strain (*P*, density n_{Pa}, where the subscript *a* indicates the presence of antibiotics); a chromosomally resistant strain (*C*, density n_{Ca}); and a chromosomally resistant strain which also carries the resistance plasmid (*CP*, density n_{CPa}). The population is therefore described as follows:

$$\begin{aligned} dn_{Pa}/dt &= n_{Pa}\left(r-\mu N-c-v\right) \\ dn_{Ca}/dt &= n_{Ca}\left(r-\mu N-c-\beta_1 n_{Pa}-\beta_1 n_{CPa}\right) \\ dn_{CPa}/dt &= n_{CPa}\left(r-\mu N-c-v+\beta_1 n_{Ca}\right)+\beta_1 n_{Pa} n_{Ca}. \end{aligned} \qquad [2]$$

We analyse this model in the same fashion as above, with the results displayed in Table 3. The strain which carries resistance on the chromosome as well as on a plasmid (*CP*) dominates. HGT is the source of the advantage of the *CP* strain allowing *CP* to increase through plasmid spread via contact between *C* and *CP* cells in addition to plasmid spread via contact between *P* and *C* cells.

Resistance plasmids: Metapopulation dynamics

Assuming within-host dynamics are rapid, using the stability and invasion conditions determined above (see Tables 2-3) we can determine the transitions that will occur between hosts in a metapopulation. These dynamics are summarized in Figure 1 (a) and Table 4. We include both hosts which are exposed to antibiotics and those which are not. We now refer to hosts as patches and denote them by the notation Q. Patches with no pathogenic bacteria, only commensals, are denoted Q_{Eo} if they are not in receipt of antibiotics and Q_{Ea} if they are (where the subscript o denotes absence of antibiotics and a the presence thereof). We assume that the antibiotic specifically targets the pathogen such that commensals are not damaged by its presence. We also include patches infected with resistant pathogens: with C (the resistance gene is on the chromosome), P (the resistance gene is on a plasmid) and CP (the resistance gene is on both the chromosome and a plasmid) strains for both antibiotic and antibiotic-free environments (Q_{Co}, Q_{Po}, Q_{CPo}, Q_{Ca}, Q_{Pa} and Q_{CPa}). Additionally, we include antibiotic-free patches infected with the wild-type pathogen (Q_{Fo}). Patches exposed to antibiotics that contain the wild-type pathogen are not included because in our within-patch dynamics we assume that wild-type cells cannot survive in the presence of antibiotics. We also assume that the plasmid is specific to this pathogenic strain such that commensals cannot be infected with it. We differentiate between three different types of transition between patches; colonization, where a particular pathogenic patch type invades a commensal patch (E_o or E_a); replacement through infection (i.e. plasmid transmission) where plasmid patches replace plasmid-free patches; and replacement through competition where pathogenic patches replace each other (without plasmid infection). The transitions between patches are outlined in Table 4 and are displayed in the full metapopulation model (equation (3)) below.

Transitions between patches are mediated by individual bacteria such that we must keep track of the total numbers of cells of a certain type during transitions between patch types. Thus the total numbers of wild-type cells, resistance plasmid carrying cells, chromosomally resistant cells and cells which carry both chromosomal and plasmid resistance are as follows:

$$N_F = Q_{Fo}\hat{n}_{Fo}$$
$$N_{Ptot} = Q_{Po}\hat{n}_{Po} + Q_{Pa}\hat{n}_{Pa} + Q_{CPo}\hat{n}_{CPo} + Q_{CPa}\hat{n}_{CPa}$$
$$N_{PP} = Q_{Po}\hat{n}_{Po} + Q_{Pa}\hat{n}_{Pa}$$
$$N_C = Q_{Co}\hat{n}_{Co} + Q_{Ca}\hat{n}_{Ca}$$
$$N_{CP} = Q_{CPo}\hat{n}_{CPo} + Q_{CPa}\hat{n}_{CPa},$$

where Q_{Fo}, Q_{Co}, Q_{Po}, Q_{CPo}, Q_{Ca}, Q_{Pa} and Q_{CPa} refer to patches. The equilibria $\hat{n}_{Fo}, \hat{n}_{Po}, \hat{n}_{Co}$ and $\hat{n}_{CPo}$ are listed in Table 2 and $\hat{n}_{Pa}, \hat{n}_{Ca}$ and $\hat{n}_{CPa}$ are listed in Table 3. N_{Ptot} refers to plasmid cells which are involved in replacement through plasmid infection (all available cells which can transmit a plasmid which can then spread in the patch) and N_{PP} refers to plasmid cells involved in colonization of patches with plasmid-carrying pathogens (where resistance is exclusively plasmid encoded). N_{Ptot} is greater than N_{PP} because it includes the total number of cells containing plasmids which can donate a plasmid to either *F* or *C* to convert them to either *P* or *CP* respectively. Colonization of commensal patches (via N_{PP}) with a certain strain requires only those cells of the genetic background of that strain. The metapopulation dynamics are as follows:

$$
\begin{aligned}
dE_o/dt &= d_{\mathrm{F}}F_o + d_{\mathrm{P}}P_o + d_{\mathrm{C}}C_o + d_{\mathrm{CP}}CP_o - (b_{\mathrm{F}}N_F + b_{\mathrm{P}}N_{PP} + b_{\mathrm{C}}N_C + b_{\mathrm{CP}}N_{CP})E_o - AE_o + ClE_a \\
dF_o/dt &= b_{\mathrm{F}}N_F E_o - d_{\mathrm{F}}F_o - \beta_{\mathrm{FP}}N_{Ptot}F_o + \alpha_{\mathrm{FC}}N_F C_o - AF_o \\
dP_o/dt &= b_{\mathrm{P}}N_{PP}E_o - d_{\mathrm{P}}P_o + \beta_{\mathrm{FP}}N_{Ptot}F_o - AP_o + ClP_a \\
dC_o/dt &= b_{\mathrm{C}}N_C E_o - d_{\mathrm{C}}C_o - \alpha_{\mathrm{FC}}N_F C_o - \beta_{\mathrm{CCP}}N_{Ptot}C_o - AC_o + ClC_a \\
dCP_o/dt &= b_{\mathrm{CP}}N_{CP}E_o - d_{\mathrm{CP}}CP_o + \beta_{\mathrm{CCP}}N_{Ptot}C_o - ACP_o + ClCP_a \\
dE_a/dt &= d_{\mathrm{F}}F_a + d_{\mathrm{P}}P_a + d_{\mathrm{C}}C_a + d_{\mathrm{CP}}CP_a - (b_{\mathrm{P}}N_{PP} + b_{\mathrm{C}}N_C + b_{\mathrm{CP}}N_{CP})E_a + AE_o - ClE_a + AF_o \\
dP_a/dt &= b_{\mathrm{P}}N_{PP}E_a - d_{\mathrm{P}}P_a + AP_o - ClP_a \\
dC_a/dt &= b_{\mathrm{C}}N_C E_a - d_{\mathrm{C}}C_a + AC_o - ClC_a - \beta_{\mathrm{CCP}}N_{Ptot}C_a \\
dCP_a/dt &= b_{\mathrm{CP}}N_{CP}E_a - d_{\mathrm{CP}}CP_a + ACP_o - ClCP_a + \beta_{\mathrm{CCP}}N_{Ptot}C_a .
\end{aligned}
\qquad [3]
$$

All pathogen patches can colonize commensal patches (at rates b_{F}, b_{P}, b_{C} and b_{CP}), and also go extinct at a certain rate (at rates d_{F}, d_{P}, d_{C} and d_{CP}). Addition of antibiotic patches (i.e. host taking antibiotics) occurs at rate A and clearance of these patches (through cessation of antibiotic use) occurs at rate Cl. The dynamics reflect the transitions we see within-patches, whereby plasmid patches (Q_{Po} and Q_{CPo}, Q_{CPa}) can replace plasmid-free patches through infection (Q_{Fo} and $Q_{Co,}$ Q_{Ca} respectively) as invasion of rare plasmid-carrying cells will lead to the spread of the plasmid throughout all the pathogen cells in the patch (as revealed in the within-patch dynamics, see Tables 2 and 3 for details).

Colonization of wild-type patches enables plasmid resistance to outcompete chromosomal resistance in the absence of antibiotics.

We can analyse the model separately in the presence and in the absence of antibiotics. In the absence of antibiotics (i.e. when Q_{Ea}, Q_{Ca}, Q_{Pa} and Q_{CPa} are zero and there is no addition or clearance of antibiotics), in order for the plasmid-carried resistant patches (Q_{Po}) to outcompete their chromosomal variants (Q_{Co}), when they are both rare, the following condition must be satisfied:

$$\left.\frac{\partial(dPo/dt)}{\partial Po}\right|_{Po\to 0,Co\to 0,Cpo\to 0} > \left.\frac{\partial(dCo/dt)}{\partial Co}\right|_{Po\to 0,Co\to 0,Cpo\to 0}.$$

As the plasmid depends on wild-type patches (to infect) we find the conditions where the wild-type is at equilibrium in the absence of other patch types ($Q_{Eo}=\frac{d_F}{b_F k}, Q_{Fo}=1-\frac{d_F}{b_F k}$) and analyse this special case. When we simplify the equations such that the rates at which commensal patches are colonized (b) as well the patch extinction rate (d) are equal for all patch-types, the above expression becomes $\beta_{FP} > \frac{bdv+(d-bk)r\alpha_{CF}}{(d-bk)(c-r+v)}$. Thus we see that the replacement (at rate β_{FP}) of wild-type patches (F_o) by plasmid patches (P_o) patches determines the success of plasmid resistance against chromosomal resistance in the absence of antibiotics. Due to long term amelioration of the costs of antibiotic resistance (Lenski 1997; zur Wiesch et al. 2010) it is reasonable to assume that over time, the rate of replacement through competition of chromosomally resistant patches with wild-type patches, α_{CF}, will eventually be negligible. Therefore the dominant force will be the ability of plasmid patches to replace wild-type pathogen patches (via β_{FP}). Wild-type patches (F_o) are a reservoir that cannot be accessed by chromosomal resistance carriers. This is seen in our simulations whereby neither plasmid nor chromosomal resistance can invade the above equilibrium from rare ($Q_{Po}=Q_{Co}=10^{-8}$) when β_{FP} is sufficiently low, leaving only commensal and wild-type patches (not shown). Assuming that all patch transitions, β_{FP} and β_{CCP}, are equal (and competition is negligible i.e. $\alpha_{CF}=0$) and sufficiently high we find that plasmids are favoured over chromosomal resistance and thus when the plasmid is equally likely to spread in both C_o and F_o patches, resistance can only be maintained in the absence of antibiotics in plasmid form (Figure 2 (a)).

There is no long-term coexistence between plasmid and chromosomal resistance.

A narrow window of opportunity exists wherein chromosomal resistance (CR) may outcompete plasmid-carried resistance (PR). This occurs when the rate at which chromosomally resistant patches are replaced by patches of both chromosomal and plasmid resistance (CP_o), β_{CCP}, is reduced relative to the rate at which wild-type patches are replaced by plasmid patches, β_{FP} (Figure 2 (b)). However, we see that plasmid patches first invade and then are subsequently outcompeted by CR. Thus the spread of CR requires the presence of PR, at least initially, when transmission levels are low and wild-type patches are replaced at a higher rate than CR patches (i.e. $\beta_{FP} > \beta_{CCP}$). When α_{CF} increases, CR can be maintained provided β_{CCP} does not increase concurrently and provided plasmid carriage exerts a relatively higher cost on their hosts than carriage of a resistance trait (an assumption that we make throughout). Therefore the initial presence of plasmid-based resistance supports its chromosomal variant in the absence of antibiotics. However, by increasing competition through α_{CF}, Q_{Co} can no longer replace Q_{Po} as competitive interactions between wild-type and CR patches deplete the level of CR. This provides new wild-type patches for PR to colonize in addition to slowing down the spread of its competitor CR.

In the absence of antibiotics we find there cannot be long-term coexistence between mobile resistance and chromosomal resistance, either in the form of both types of patches (Q_{Co} and Q_{Po}) or in the form of patches where pathogens carry both forms of resistance (Q_{CPo}).

Low addition and high clearance of antibiotics favours plasmid carriers.

We find that PR is rapidly favoured when antibiotics are added at a low rate. Plasmid patches are boosted by replacement through infection of wild-type patches. Increasing the rate of addition of antibiotics (Figure 3) creates sufficient selection pressure to maintain chromosomal resistance in the form of CP patches (hereafter referred to as double resistance (CRPR)).

Increasing the rate of clearance of antibiotics (*Cl*) from the metapopulation sustains coexistence of PR (plasmid-carried resistance) and CRPR, now also maintained in antibiotic-free patches as well. Unless the rate at which chromosomally resistant patches are replaced by patches of both chromosomal and plasmid resistance (β_{CCP}) is reduced, CR is not maintained in the long-term in the presence of antibiotics. Instead chromosomal resistance is present in the form of CRPR. This contrasts with the results in the absence of antibiotics where such coexistence was not seen.

Plasmid competition: Within-patch population dynamics

To reflect the fact that different plasmid types are abundant in nature we now introduce a second plasmid to examine the effect of plasmid competition on our model dynamics. We assume that this second plasmid, which we refer to as *B*, is incompatible with the antibiotic-resistance plasmid *P* i.e. *P* and *B* cannot stably coexist in the same cell (Novick 1987).We assume the *B* plasmid can infect the same hosts as the *P* plasmid (i.e. *F* and *C*), but as a result of incompatibility between the two plasmids, cells can only carry either *B* or *P* but not both. We assume that *B* does not carry an antibiotic resistance gene (what we term a benign

plasmid) and therefore incurs no cost c on the host but because it continues to use host resources to replicate it retains the virulence cost v. Plasmid P is transmitted at rate β_1 as before and plasmid B is transmitted at rate β_2. As B is not resistant to antibiotics it will compete with P only in antibiotic-free patches. We will use this model to investigate the effect of plasmid competition on the advantage of plasmid-carried resistance over chromosomally-based resistance. Densities are defined as for equations (1) and (2) with the addition of B in antibiotic-free patches (density n_{Bo}) as well as chromosomally resistant cells infected with the benign plasmid (CB, density n_{CBo}) which are retained in the presence of antibiotics (density n_{CBa}). In the absence of antibiotics the within-host dynamics are as follows:

$$\begin{aligned}
dn_{Fo}/dt &= n_{Fo}\left(r-\mu N-\beta_1 n_{Po}-\beta_1 n_{CPo}-\beta_2 n_{Bo}-\beta_2 n_{CBo}\right)\\
dn_{Po}/dt &= n_{Po}\left(r-\mu N-c-v+\beta_1 n_{Fo}\right)+\beta_1 n_{CPo} n_{Po}\\
dn_{Bo}/dt &= n_{Bo}\left(r-\mu N-v+\beta_2 n_{Fo}\right)+\beta_2 n_{CBo} n_{Po}\\
dn_{Co}/dt &= n_{Co}\left(r-\mu N-c-\beta_1 n_{Po}-\beta_1 n_{CPo}-\beta_2 n_{Bo}-\beta_2 n_{CBo}\right)\\
dn_{CPo}/dt &= n_{CPo}\left(r-\mu N-c-v+\beta_1 n_{Co}\right)+\beta_1 n_{Po} n_{Co}\\
dn_{CBo}/dt &= n_{CBo}\left(r-\mu N-c-v+\beta_2 n_{Co}\right)+\beta_2 n_{Bo} n_{Co}.
\end{aligned} \qquad [4]$$

When antibiotics are added the dynamics are simplified to:

$$\begin{aligned}
dn_{Pa}/dt &= n_{Pa}\left(r-\mu N-c-v\right)\\
dn_{Ca}/dt &= n_{Ca}\left(r-\mu N-c-\beta_1 n_{Pa}-\beta_1 n_{CPa}-\beta_2 n_{CBa}\right)\\
dn_{CPa}/dt &= n_{CPa}\left(r-\mu N-c-v+\beta_1 n_{Ca}\right)+\beta_1 n_{Pa} n_{Ca}\\
dn_{CBa}/dt &= n_{CBa}\left(r-\mu N-c-v+\beta_2 n_{Ca}\right).
\end{aligned} \qquad [5]$$

The equilibria for these models and their stability conditions are listed in Tables 2 and 3 as before. We use the same techniques as for the single plasmid scenario to model plasmid competition in a metapopulation (summarized in Figure 1 (b)). The metapopulation dynamics (as described in Table 4) are therefore:

$$
\begin{aligned}
dE_o/dt &= d_{\mathrm{F}}F_o + d_{\mathrm{P}}P_o + d_{\mathrm{B}}B_o + d_{\mathrm{C}}C_o + d_{\mathrm{CP}}CP_o + d_{\mathrm{CB}}CB_o - AE_o + ClE_a \\
&\quad - (b_{\mathrm{F}}N_F + b_{\mathrm{P}}N_{PP} + b_{\mathrm{B}}N_{BB} + b_{\mathrm{C}}N_C + b_{\mathrm{CP}}N_{CP} + b_{\mathrm{CB}}N_{CB})E_o \\
dF_o/dt &= b_{\mathrm{F}}N_F E_o - d_{\mathrm{F}}F_o - \beta_{\mathrm{FP}}N_{Ptot}F_o - \beta_{\mathrm{FB}}N_{Btot}F_o + \alpha_{\mathrm{CF}}N_F C_o - AF_o \\
dP_o/dt &= b_{\mathrm{P}}N_{PP}E_o - d_{\mathrm{P}}P_o + \beta_{\mathrm{FP}}N_{Ptot}F_o - AP_o + ClP_a - \alpha_{\mathrm{PB}}N_{BB}P_o \\
dB_o/dt &= b_{\mathrm{B}}N_{BB}E_o - d_{\mathrm{B}}B_o + \beta_{\mathrm{FB}}N_{Btot}F_o - AB_o + \alpha_{\mathrm{PB}}N_{BB}P_o + \alpha_{\mathrm{CB}}N_{BB}C_o + \alpha_{\mathrm{CBB}}N_{BB}CB_o + \alpha_{\mathrm{CPB}}N_{BB}CP_o \\
dC_o/dt &= b_{\mathrm{C}}N_C E_o - d_{\mathrm{C}}C_o - \alpha_{\mathrm{CF}}N_F C_o - \beta_{\mathrm{CP}}N_{Ptot}C_o - \beta_{\mathrm{CB}}N_{Btot}C_o - AC_o + ClC_a - \alpha_{\mathrm{CB}}N_{BB}C_o \\
dCP_o/dt &= b_{\mathrm{CP}}N_{CP}E_o - d_{\mathrm{CP}}CP_o + \beta_{\mathrm{CP}}N_{Ptot}C_o - ACP_o + ClCP_a - \alpha_{\mathrm{CPB}}N_{BB}CP_o \\
dCB_o/dt &= b_{\mathrm{CB}}N_{CB}E_o - d_{\mathrm{CB}}CB_o + \beta_{\mathrm{CB}}N_{Btot}C_o - ACB_o + ClCB_a - \alpha_{\mathrm{CBB}}N_{BB}CB_o \\
dE_a/dt &= d_{\mathrm{F}}F_a + d_{\mathrm{P}}P_a + d_{\mathrm{C}}C_a + d_{\mathrm{CP}}CP_a + d_{\mathrm{CB}}CB_a - (b_{\mathrm{P}}N_{PP} + b_{\mathrm{C}}N_C + b_{\mathrm{CP}}N_{CP} + b_{\mathrm{CB}}N_{CB})E_a \\
&\quad + AE_o - ClE_a + AF_o + AB_o \\
dP_a/dt &= b_{\mathrm{P}}N_{PP}E_a - d_{\mathrm{P}}P_a + AP_o - ClP_a \\
dC_a/dt &= b_{\mathrm{C}}N_C E_a - d_{\mathrm{C}}C_a + AC_o - ClC_a - \beta_{\mathrm{CP}}N_P C_a - \beta_{\mathrm{CB}}N_{Btot}C_a \\
dCP_a/dt &= b_{\mathrm{CP}}N_{CP}E_a - d_{\mathrm{CP}}CP_a + ACP_o - ClCP_a + \alpha_{\mathrm{CP}}N_{Ptot}C_a \\
dCB_a/dt &= b_{\mathrm{CB}}N_{CB}E_a - d_{\mathrm{CB}}CB_a + ACB_o - ClCB_a + \alpha_{\mathrm{CB}}N_{Btot}C_a .
\end{aligned}
\qquad [6]
$$

The total numbers of cell types N_{Ptot}, N_{PP}, N_F, N_C and N_C are calculated as they were in the original metapopulation model (equation (3)) with the additional values of N_{Btot} (benign plasmid carrying cells involved in infection) and N_{BB} referring to *B* plasmid cells involved in colonization where:

$$
\begin{aligned}
N_{Btot} &= Q_{Bo}\hat{n}_{Bo} + Q_{CBo}\hat{n}_{CBo} + Q_{CBa}\hat{n}_{CBa}, \\
N_{BB} &= Q_{Bo}\hat{n}_{Bo}.
\end{aligned}
$$

Plasmid competition suppresses resistance in the absence of antibiotics.

We find that when a benign plasmid can successfully outcompete a resistance plasmid, in the absence of antibiotic treatment, that there is an overall suppression of both chromosomal resistance (CR) and plasmid resistance (PR) (Figure 4 (a)). However upon addition of antibiotics we find that plasmid competition favours the spread of coexistence in the form of double resistance, CRPR, (Figure 4(c)) or solely chromosomal resistance, CR, (Figure 4 (d)), unless addition of antibiotics occurs at a very low rate in which case PR is briefly successful before patches infected with the benign plasmid carrying pathogenic strain replace plasmid-carrying pathogen patches (not shown).

6.4 Discussion

Our results confirm that antibiotic resistance genes will be selected to be carried on plasmids. We have shown that plasmid-carried resistance (PR) is extensively favoured over chromosomal-carried resistance (CR). This is supported by previous results regarding within-population dynamics: plasmids are likely to be most successful in this scenario as sufficiently high rates of transfer can maintain them even when they do not carry genes which are currently useful (e.g. Bahl et al. 2007). In the presence of antibiotics, heterogeneity in the environment (i.e. more variety in patch types, as influenced by antibiotic addition and clearance) favours the spread of plasmids.

Chromosomal resistance (CR) may spread when there is a lower chance of double resistance (CRPR) replacing CR patches (β_{CCP}) than there is of (plasmid-carried resistance) PR replacing wild-type pathogen patches (β_{FP}). The rate of plasmid invasion into chromosomal patches

(described by β_{CCP} in the model) is lower than the rate of plasmid invasion into wild-type patches (β_{FP} in the model) when the different types of resistance are be clustered, making it unlikely that the two resistance types will encounter each other. This could occur, for example, in hospital wards, where resistance may dominate in one form (plasmid or chromosome-based) and co-occur with resistance-free cells when new patients enter the ward. Under these circumstances PR and CR patches co-occur at a lower frequency than PR and wild-type patches. Our results show that, in such a setting, PR will invade but will eventually be replaced by CR (Figure 2). This demonstrates that plasmids act as drivers of antibiotic resistance in the population, even when they do not persist in the long-term. While we do not observe the coexistence of PR and CR in the absence of antibiotic treatment, the removal of wild-type patches creates a vacuum allowing for chromosomal resistance to spread. This occurs even if the transfer of plasmids into chromosomal patches (β_{CCP}) is on a par with the rate of transfer of plasmids into wild type plasmids (β_{FP}). In this case, then chromosomal resistance is maintained in the form of CRPR.

We see that plasmid-carried resistance will be outcompeted by competing non-resistance plasmids, and both plasmid and chromosomal resistance suppressed in the absence of antibiotic treatment. However, when treatment is introduced, chromosomal resistance will be able to persist. Competition between two incompatible plasmids removes the advantage of horizontal gene transfer (HGT) as a means of spread when competing for hosts with a less costly plasmid (Mc Ginty et al. 2011). We may therefore expect a chromosomal variant to be favoured when the resistance plasmid competes with an incompatible benign plasmid. Previous studies have shown that resistance plasmids outcompete benign plasmids when dosage of antibiotics is higher, in addition to outcompeting cells carrying chromosomal resistance under longer intervals between antibiotic treatments (Svara and Rankin 2011). Our

results show that competition with a non-resistance plasmid can diminish the number of resistance plasmids, and thus may inadvertently favour chromosomal resistance. Aside from plasmid competition, the loss of plasmids at cell division through segregation may contribute to the maintenance of CR in the population (as chromosomal resistance is not lost in this fashion). This may be particularly true in the case of CRPR, where we would expect that if resistance is selected for and already present on the chromosome, the costly plasmid may be lost over time.

While plasmids appear to be key drivers in the spread of antibiotic resistance (DeNap et al. 2004; Bennett 2008; Williams and Hergenrother 2008; Hawkey and Jones 2009), there are many examples of chromosomally-based resistance (e.g. Piddock 2006), and mutation remains an important means of generating new chromosomal resistance. It has been predicted that consistent positive selection on beneficial traits (such as antibiotic resistance) will lead to these genes being integrated into the chromosome (Bergstrom et al. 2000), potentially resulting in the loss of the resistance-carrying plasmid in the population (Lili et al. 2007). We do not examine the mechanisms by which plasmid-encoded genes may be incorporated into the chromosome, though factors such as recombination are likely to play an important role in the maintenance of resistance genes. However, the incorporation of PR into the chromosome appears to be rare, and can occur in the absence of positive selection (i.e. the absence of antibiotics) (Modi et al. 1992). There are many studies when involving positive selection where the mobile resistance traits are not incorporated into the chromosome (e.g. Bouma and Lenski 1988; Turner et al. 1998; Dionisio et al. 2005) suggesting chromosomal incorporation of useful traits it is not as strong a force as predicted.

Plasmids tend to be lost in the absence of positive selection unless they have very high transfer rates (Levin 1993; Bergstrom et al. 2000). Plasmids can sacrifice accessory genes in a bid to reduce cost of carriage. We may therefore expect the loss of antibiotic resistance genes over time in the absence of selection for resistance (Modi et al. 1992; Subbiah et al. 2011). However, sufficiently high between-host transmission is required to sustain costly plasmids (Levin 1993; Bahl et al. 2007; Lili et al. 2007) and the costs of resistance can be also be ameliorated (Andersson and Hughes 2010). For example, a costly streptomycin-resistance allele at one locus can be ameliorated by compensatory mutations at a different locus in *Salmonella typhimurium* (Maisnier-Patin et al. 2007). This reduction in cost could facilitate the maintenance of a chromosomal variant, meaning that an overall reduction in antibiotic use will not necessarily result in a loss of antibiotic resistance from the population. Indeed, persistence of resistance despite reduction in antibiotic use is known to occur (Enne et al. 2001; Bean et al. 2005).

We assume that both CR and PR represent the same resistance mechanism. However chromosomal resistance and plasmid resistance are often fundamentally different (Martinez et al. 2007). Genes that are acquired through plasmids usually have an environmental origin (Martinez et al. 2007) whereas chromosomal resistance occurs through genes that are already encoded in the bacterial genome and that can confer resistance through mutation (Martinez and Baquero 2000) or activation by different mobile elements (Olliver et al. 2005). Plasmids' host ranges frequently include bacteria of different genera and habitats giving them the opportunity to acquire diverse genes from many sources, leading to plasmid-encoded resistance being observed clinically in all major classes of antibiotics (DeNap et al. 2004) including β-lactams and tetracyclines (Nordmann and Naas 1997; Billington et al. 2002; Philippon et al. 2002). The mobile nature of plasmids has allowed them to carry multiple

resistance genes at once (Walsh 2006), for example in the case of vancomycin-resistant enterococci (VRE) and *Staphylococcus aureus* (VRSA) (Weigel et al. 2003). The trend towards increasingly large and complex regions of mobile antibiotic resistance has been noted elsewhere (Stokes and Gillings 2011).

It is clear that plasmids are drivers of resistance, and even the process of conjugative transfer itself can promote antibiotic resistance by activating responses that lead to expression of resistance genes (Baharoglu et al. 2010). Our model demonstrates that we should expect plasmid-encoded resistance to dominate, confirming frequent empirical observations of plasmid-based antibiotic resistance (e.g. Nordmann and Naas 1997; Billington et al. 2002; Johnsen et al. 2002; Philippon et al. 2002). However focusing on plasmids may provide an inspiration for new techniques to limit the spread of antibiotic resistance genes. Plasmids have been described as the "Achilles heel" of antibiotic resistance (Williams and Hergenrother 2008), and the exploitation of incompatibility mechanisms has been proposed as a method to reduce the spread of antibiotic resistance plasmids (DeNap et al. 2004). Attempts to stem the rising tide of antibiotic resistance must therefore focus on two strategies: limiting within-bacterial population spread by targeting plasmids themselves (Williams and Hergenrother 2008), and focusing on reducing the rate of spread of pathogens carrying plasmid-encoded resistance to new hosts.

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6.5 Tables

Table 1. Parameters for within-patch population dynamics.

Parameter	Definition
N	Total population size
c	Cost of resistance
v	Cost of plasmid carriage
k	Population carrying capacity
β_1	Rate at which cells are infected by resistance plasmid
β_2	Rate at which cells are infected by benign competitor plasmid (without resistance gene)
r	Intrinsic growth rate
μ	Density-dependent death rate ($\mu=r/k$)

Table 2. Non-trivial within-patch equilibria and stability conditions in the absence of antibiotics. Transitions indicate where the focal equilibrium is invaded and by what strain. "Stable" equilibria cannot be successfully invaded by the other strains. Coexistence equilibria are unstable in this model (have one or more non-negative eigenvalues) and are not displayed. Wild type strains are denoted F, strains carrying resistance encoded by a plasmid, by the chromosome or by both a plasmid and the chromosome are denoted P, C and CP respectively. Strains carrying the competitor plasmid are denoted with B and chromosomally resistant strains carrying the competitor plasmid are denoted CB. Arrows indicate invasion i.e. x→y indicates that y invades x. Continued overleaf.

* In absence of plasmid competition (i.e. the absence of B and CB)

**Transition C→B can happen under the condition $v<c$, which we assume does not occur.

Equilibrium		Transition	Condition
Pure F ($\hat{n}_{\mathrm{Fo}}$)	$\hat{n}_{\mathrm{Fo}}=k, \hat{n}_{\mathrm{Po}}=0, \hat{n}_{\mathrm{Co}}=0, \hat{n}_{\mathrm{CPo}}=0$ $\hat{n}_{\mathrm{Bo}}=0, \hat{n}_{\mathrm{CBo}}=0$	F→P	$c+v<k\beta_1$
		F→B	$v<k\beta_2$
Pure P ($\hat{n}_{\mathrm{Po}}$)	$\hat{n}_{\mathrm{Fo}}=0, \hat{n}_{\mathrm{Po}}=k(r-c-v)/r, \hat{n}_{\mathrm{Co}}=0,$ $\hat{n}_{\mathrm{CPo}}=0, \hat{n}_{\mathrm{Bo}}=0, \hat{n}_{\mathrm{CBo}}=0$	Stable*	
		P→B	$c>0$
Pure C ($\hat{n}_{\mathrm{Co}}$)	$\hat{n}_{\mathrm{Fo}}=0, \hat{n}_{\mathrm{Po}}=0, \hat{n}_{\mathrm{Co}}=k-ck/r,$ $\hat{n}_{\mathrm{CPo}}=0, \hat{n}_{\mathrm{Bo}}=0, \hat{n}_{\mathrm{CBo}}=0$	C→F	$c>0$
		C→CP	$k\beta_1>rv/(r-c)$
		C→CB	$k\beta_2>rv/(r-c)$
		C→B**	$v<c$

Table 2. continued.

Equilibrium		Transition	Condition
Pure CP ($\hat{n}_{\mathrm{CPo}}$)	$\hat{n}_{\mathrm{Fo}}=0,\hat{n}_{\mathrm{Po}}=0,\hat{n}_{\mathrm{Co}}=0,$ $\hat{n}_{\mathrm{CPo}}=k(r-v-c)/r,\hat{n}_{\mathrm{Bo}}=0,\hat{n}_{\mathrm{CBo}}=0$	Stable*	
		CP→B	$c>0$
Pure B ($\hat{n}_{\mathrm{Bo}}$)	$\hat{n}_{\mathrm{Fo}}=0,\hat{n}_{\mathrm{Po}}=0,\hat{n}_{\mathrm{Co}}=0,\hat{n}_{\mathrm{CPo}}=0,$ $\hat{n}_{\mathrm{Bo}}=k-(kv/r),\hat{n}_{\mathrm{CBo}}=0$	Stable	
Pure CB ($\hat{n}_{\mathrm{CBo}}$)	$\hat{n}_{\mathrm{Fo}}=0,\hat{n}_{\mathrm{Po}}=0,\hat{n}_{\mathrm{Co}}=0,\hat{n}_{\mathrm{CPo}}=0,\hat{n}_{\mathrm{Bo}}=0,$ $\hat{n}_{\mathrm{CBo}}=k(r-v-c)/r$	Unstable	
		CB→B	$c>0$

Table 3. Non-trivial within-patch equilibria and stability conditions in the presence of antibiotics. Transitions indicate where the focal equilibrium is invaded and by what strain. "Stable" equilibria cannot be successfully invaded by the other strains. Coexistence equilibria are unstable in this model (have one or more non-negative eigenvalues) and are not displayed. Wild type strains are denoted F, strains carrying resistance encoded by a plasmid, by the chromosome or by both a plasmid and the chromosome are denoted P, C and CP respectively. Strains infected with a non-resistance carrying plasmid are denoted B. Chromosomally resistant strains carrying the competitor plasmid are denoted CB. Arrows indicate invasion i.e. x→y indicates that y invades x.

* In absence of plasmid competition

Equilibrium		Transition	Condition
Pure P ($\hat{n}_{Pa}$)	$\hat{n}_{Pa} = -(k\,(c-r+v))/r, \hat{n}_{Ca} = 0,$ $\hat{n}_{CPa} = 0, \hat{n}_{CBa} = 0$	Stable	
Pure C ($\hat{n}_{Ca}$)	$\hat{n}_{Pa} = 0, \hat{n}_{Ca} = k - ck/r,$ $\hat{n}_{CPa} = 0, \hat{n}_{CBa} = 0$	C→CP	$k\beta_1 > rv/(r-c)$
		C→CB	$k\beta_2 > rv/(r-c)$
Pure CP ($\hat{n}_{CPa}$)	$\hat{n}_{Fa} = 0, \hat{n}_{Pa} = 0, \hat{n}_{Ca} = 0,$ $\hat{n}_{CPa} = k(r-v-c)/r, \hat{n}_{CBa} = 0$	Stable	
Pure CB ($\hat{n}_{CBa}$)	$\hat{n}_{Fa} = 0, \hat{n}_{Pa} = 0, \hat{n}_{Ca} = 0,$ $\hat{n}_{CPa} = 0, \hat{n}_{CBa} = k(r-v-c)/r$	Stable	

Table 4. Patch transitions in the metapopulation (continued overleaf). Where $x = \{o, a\}$: E_x = empty patches (patches not infected with the pathogen), F_o = wild-type patches, C_x = patches of chromosomally encoded resistance, P_x = patches of plasmid encoded resistance, CP_x = patches of chromosomal and plasmid encoded resistance, B_o= patches of carriers of benign plasmid, CB_x = patches of chromosomally resistant carriers of the benign plasmid. Arrows indicate colonization/replacement i.e. x→y indicates that y replaces x. Continued overleaf.

Transition	Process	Rate
Colonization of commensal (non-pathogen) patches	$E_o \rightarrow F_o$	b_F
	$E_o \rightarrow P_o$, $E_a \rightarrow P_a$	b_P
	$E_o \rightarrow B_o$	b_B
	$E_o \rightarrow C_o$, $E_a \rightarrow C_a$	b_C
	$E_o \rightarrow CP_o$, $E_a \rightarrow CP_a$	b_{CP}
	$E_o \rightarrow CB_o$	b_{CB}
Clearance of pathogenic infection from patches (patch extinction)	$F_o \rightarrow E_o$	d_F
	$P_o \rightarrow E_o$, $P_a \rightarrow E_a$	d_P
	$B_o \rightarrow E_o$	d_B
	$C_o \rightarrow E_o$, $C_a \rightarrow E_a$	d_C
	$CP_o \rightarrow E_o$, $CP_a \rightarrow E_a$	d_{CP}
	$CB_o \rightarrow E_o$, $CB_a \rightarrow E_a$	d_{CB}
Addition of antibiotics	$E_o \rightarrow E_a$	A
	$F_o \rightarrow E_a$, $B_o \rightarrow E_a$	
	$P_o \rightarrow P_a$	
	$C_o \rightarrow C_a$	
	$CP_o \rightarrow CP_a$, $CB_o \rightarrow CB_a$	

Transition	Process	Rate
Clearance of antibiotics	$E_a \rightarrow E_o$	Cl
	$P_a \rightarrow P_o$	
	$C_a \rightarrow C_o$	
	$CP_a \rightarrow CP_o$, $CB_a \rightarrow CB_o$	
Replacement (through competition) of C by F or B	$C_o \rightarrow F_o$	α_{CF}
	$C_o \rightarrow B_o$	α_{CB}
Replacement (through plasmid infection) of C_o by CP_o (and C_a by CP_a)	$C_o \rightarrow CP_o$	β_{CCP}
	$C_a \rightarrow CP_a$	
Replacement (through plasmid infection) of C_o or CB_o or CB_a	$C_o \rightarrow CB_o$	β_{CCB}
	$C_a \rightarrow CB_a$	
Replacement (through competition) of CB_o by B_o	$CB_o \rightarrow B_o$	α_{CBB}
Replacement (through competition) of CP_o by B_o	$CP_o \rightarrow B_o$	α_{CPB}

6.6 Figure legends

Figure 1. Metapopulation dynamics of plasmid and chromosomal antibiotic resistance.

Panel (a) Plasmid versus chromosomal antibiotic resistance. Panel (b) The effects of plasmid competition. White patches are antibiotic-free, antibiotic patches are shaded grey. Solid lines indicate replacement by infection (described with β parameters in Table 4). Grey broken lines indicate addition and clearance of antibiotics. Black dot-dashed lines indicate replacement by a competitor (described with α parameters in Table 4). In panel (a) black dotted lines indicate colonization of commensal patches and pathogen patch extinction (not shown in panel (b) for clarity). Where $x = \{o, a\} : E_x$ = empty patches (patches not infected with the pathogen), F_o = wild-type patches, C_x = patches of chromosomally encoded resistance, P_x = patches of plasmid encoded resistance, CP_x =patches of chromosomal and plasmid encoded resistance, B_o= patches of carriers of benign plasmid, CB_x = patches of chromosomally resistant carriers of the benign plasmid. Arrow heads indicate the direction of the transition e.g. $F_o \rightarrow P_o$ indicates that F_o is replaced with P_o through plasmid infection.

Figure 2. In the absence of antibiotics, plasmid carried resistance dominates if wild-type and chromosomally resistant patches are replaced by plasmid patches at the same rate. If chromosomally resistant patches are less likely to interact with plasmid patches then chromosomal resistance can spread.

Horizontal axis depicts units of time (in hours). Vertical axis depicts the proportion of patches. Parameters: all rates of colonization of commensal patches (b) =0.2, all patch extinction rates (d) =0.1, r=2,v=0.05,c=0.0001, k=10,000, α_{FC} =0.

Panel (a) Wild-type and chromosomally resistant patches are replaced by plasmid patches at the same rate: β_{FP} =0.00001, β_{CCP}=0.00001. Panel (b) Chromosomally resistant patches are

replaced by plasmid patches at a lower rate than wild-type patches are replaced by plasmid patches: β_{FP} =0.00001, β_{CCP} =0.0000001.

Initial conditions: $Q_{Eo}=d_F/(B_F\ k)$, $Q_{Fo}=1 - d_F/(B_F\ k)$, $Q_{Co}=Q_{Po}=10^{-8}$, $Q_{CPo}=0$. Patch types: grey dot-dashed line = Q_{Eo}, grey dotted line = Q_{Fo}, black solid line = Q_{Po}, red solid line = Q_{Co}, blue solid line = Q_{CPo}, magenta dotted line=sum of patch proportions.

Figure 3. Increasing the addition of antibiotics rapidly favours coexistence of the two types of resistance in the form of patches of chromosomal- and plasmid-encoded antibiotic resistance.

Horizontal axis depicts units of time (in hours). Vertical axis depicts the proportion of patches. Parameters: all rates of colonization of commensal patches (b) =0.2, all patch extinction rates (d) =0.1, r=2,v=0.05,c=0.001, k=10,000, α_{FC} =0, wild-type and chromosomally resistant patches are replaced by plasmid patches at the same rate: β_{FP} =0.00001, β_{CCP}=0.00001, there is addition but not clearance of antibiotics: A=0.5, Cl=0. Initial conditions: $Q_{Eo}=d_F/(B_F\ k)$, $Q_{Fo}=1 - d_F/(B_F\ k)$, $Q_{Co}=Q_{Po}=10^{-8}$, $Q_{CPo}=Q_{Ea}= Q_{Pa}= Q_{Ca}= Q_{CPa}=0$. Patch types: grey solid line = Q_{Eo}, grey dashed line = Q_{Ea}, grey dotted line = Q_{Fo}, black solid line = Q_{Po}, black dashed line = Q_{Pa}, red solid line = Q_{Co}, red dashed line = Q_{Ca}, blue solid line = Q_{CPo}, blue dashed line = Q_{CPa}, magenta dotted line=sum of patch proportions.

Figure 4. Plasmid competition in a metapopulation.

Horizontal axis depicts units of time (in hours). Vertical axis depicts the proportion of patches. Parameters: all rates of colonization of commensal patches (b) =0.2, all patch extinction rates (d) =0.1, r=2,v=0.05,c=0.001, k=10,000.

Panels (a) and (b) initial conditions: $Q_{Eo}=d_F/(B_F\ k)$, $Q_{Fo}=1 - d_F/(B_F\ k)$, $Q_{Co}=Q_{Po}= Q_{Bo}=10^{-8}$, $Q_{Ea}=Q_{CPo}=Q_{Ea}= Q_{Pa}= Q_{Ca}= Q_{CPa}=0$.

Panels (c) and (d) initial conditions: $Q_{Eo}=A+d_F/(B_F\ k)$, $Q_{Fo}= - (A^2+A\ Cl+A\ d_F +Cl\ d_F -B_F\ Cl\ k)/(AB_F\ k+ B_F\ Cl\ k)$, $Q_{Ea}=A/A+Cl$, $Q_{Co}=Q_{Po}= Q_{Bo}=10^{-8}$, $Q_{CPo}=Q_{Ea}= Q_{Pa}= Q_{Ca}= Q_{CPa}=0$.

Patch types: grey solid line = Q_{Eo}, grey dashed line = Q_{Ea}, grey dotted line = Q_{Fo}, black solid line = Q_{Po}, black dashed line = Q_{Pa}, red solid line = Q_{Co}, red dashed line = Q_{Ca}, blue solid line = Q_{CPo}, blue dashed line = Q_{CPa}, green solid line = Q_{Bo}, magenta solid line = Q_{CBo}, magenta dashed line = Q_{CBa}, black dot-dashed line=sum of patch proportions.

Patches (a) and (b): A = Cl =0, patches (c) and (d) A = 0.6, Cl=0.5

(a) In the absence of antibiotics: when competitive interactions are negligible and transmission rates are equal then the benign plasmid dominates. β_{FP} =0.00001, β_{FB} =0.00001, β_{CCP}=0.00001, β_{CCB}=0.00001, $\alpha_{CBB}= \alpha_{CB}= \alpha_{CF}= \alpha_{PB}= \alpha_{CPB}=0$.

(b) Decreasing the rate at which chromosomal patches interact with and are replaced by plasmid patches leads to chromosomal resistance: β_{FP} =0.00001, β_{FB} =0.00001, β_{CCP}=0.0000001, β_{CCB}=0.0000001, $\alpha_{CBB}= \alpha_{CB}= \alpha_{CF}= \alpha_{PB}= \alpha_{CPB}=0$.

(c) Addition of antibiotics supports coexistence of resistance types in the form of patches of both chromosomal and plasmid resistance (CRPR) when wild-type and chromosomally resistant patches are replaced by plasmid patches at the same rate: β_{FP} =0.00001, β_{FB} =0.00001, β_{CCP}=0.00001, β_{CCB}=0.00001, $\alpha_{CF}=\alpha_{CB}=0$, $\alpha_{CBB} = \alpha_{PB}= \alpha_{CPB}=0.00001$.

(d) Increasing plasmid competition supports chromosomal resistance in the presence of antibiotics: β_{FP} =0.00001, β_{FB} =0.00001, β_{CCP}=0.00001, β_{CCB}=0.00001, $\alpha_{CF}=\alpha_{CB}=0$, $\alpha_{CBB} = \alpha_{PB}= \alpha_{CPB}=0.001$.

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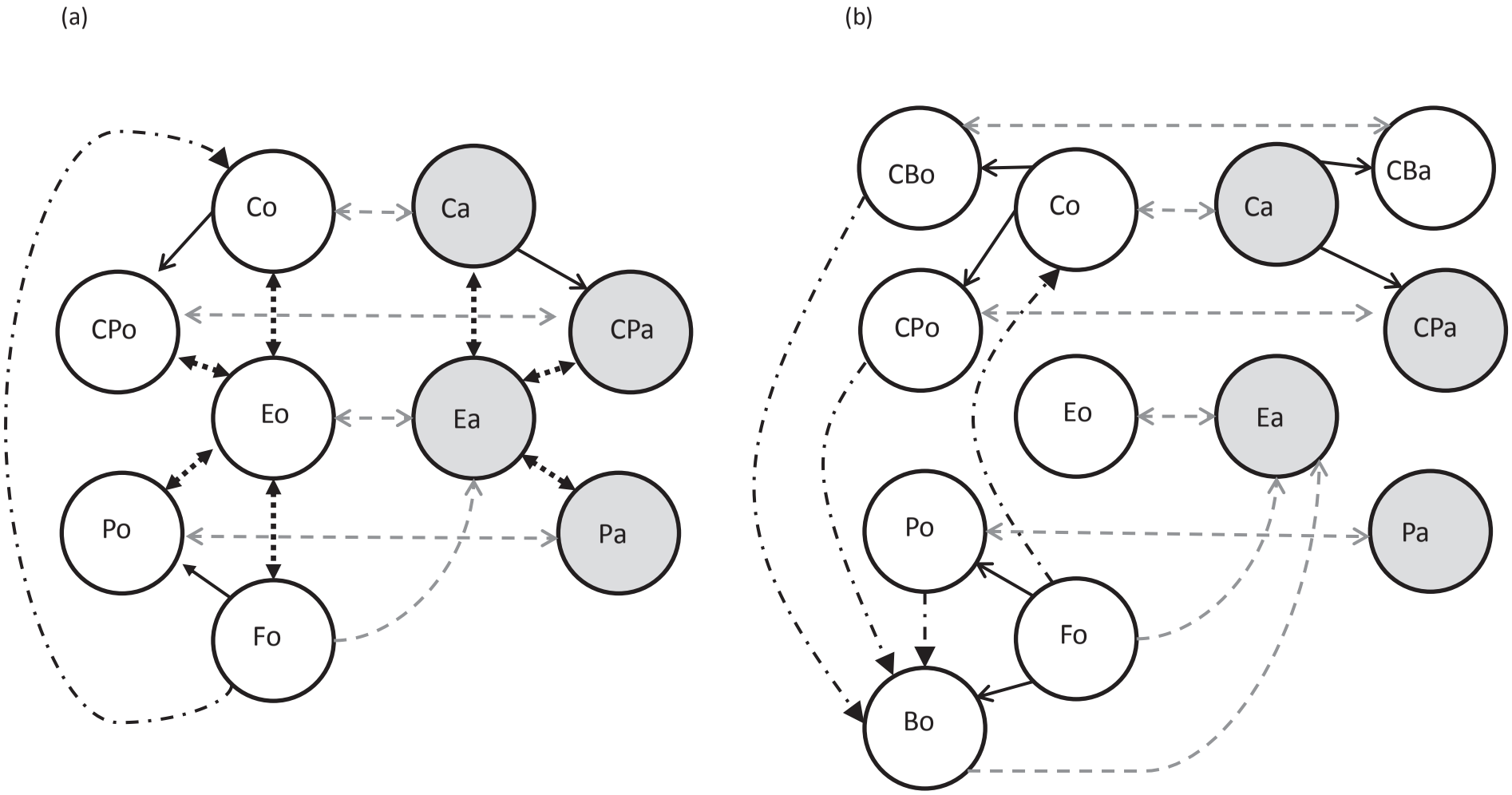
(a)
Co
Ca
CPo
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Eo
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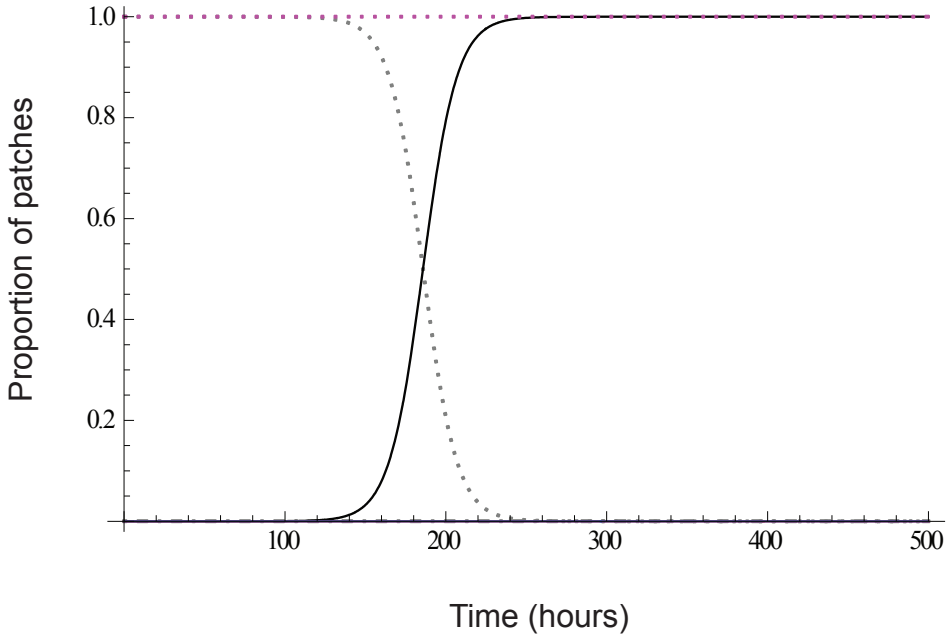
Bo

Figure 1

(a)


1.0
0.8
0.6
0.4
0.2
Proportion of patches
100
200
300
400
500
Time (hours)

(b)

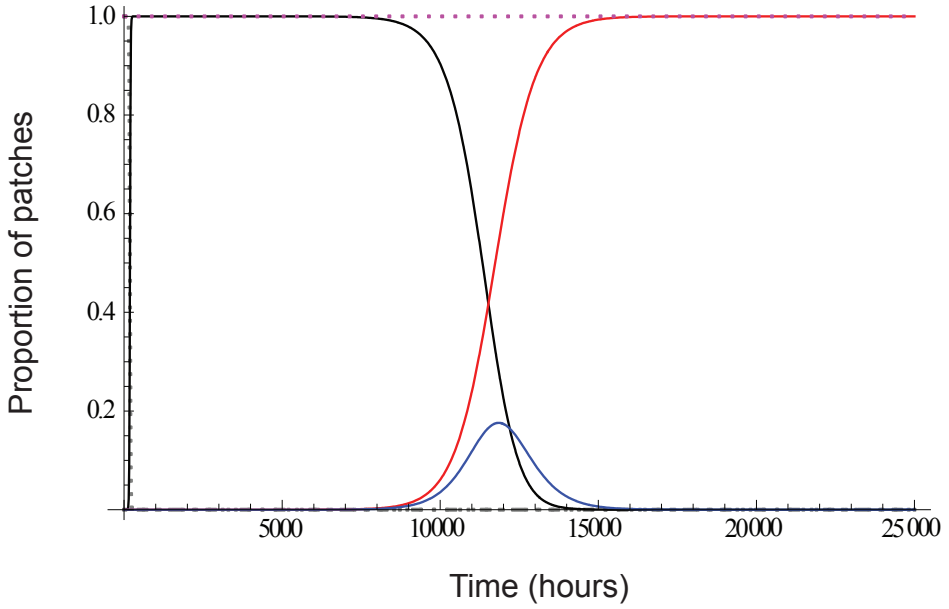

1.0
0.8
0.6
0.4
0.2
Proportion of patches
5000
10000
15000
20000
25000
Time (hours)

Figure 2

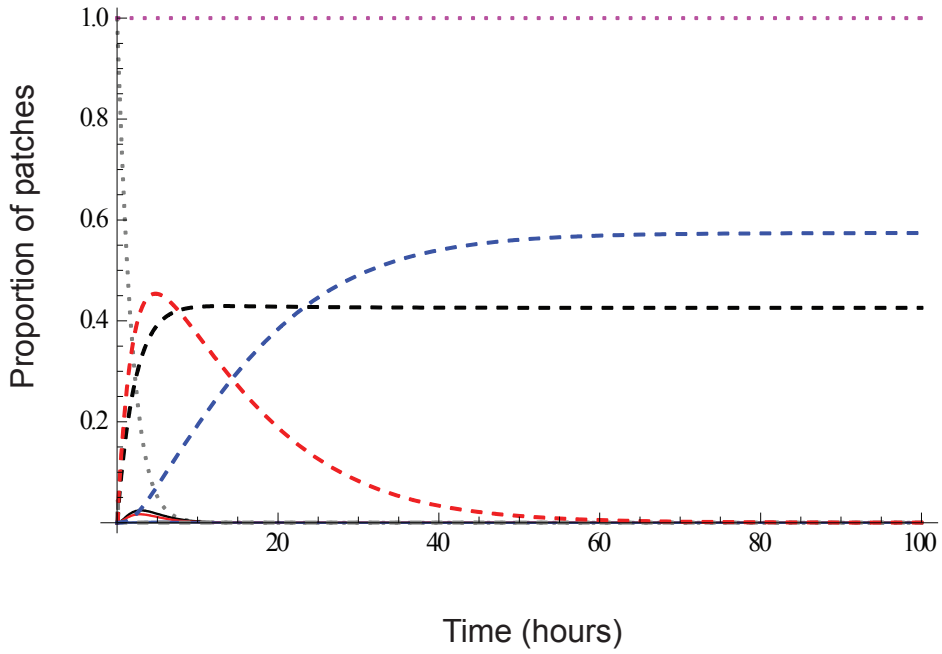


Figure 3

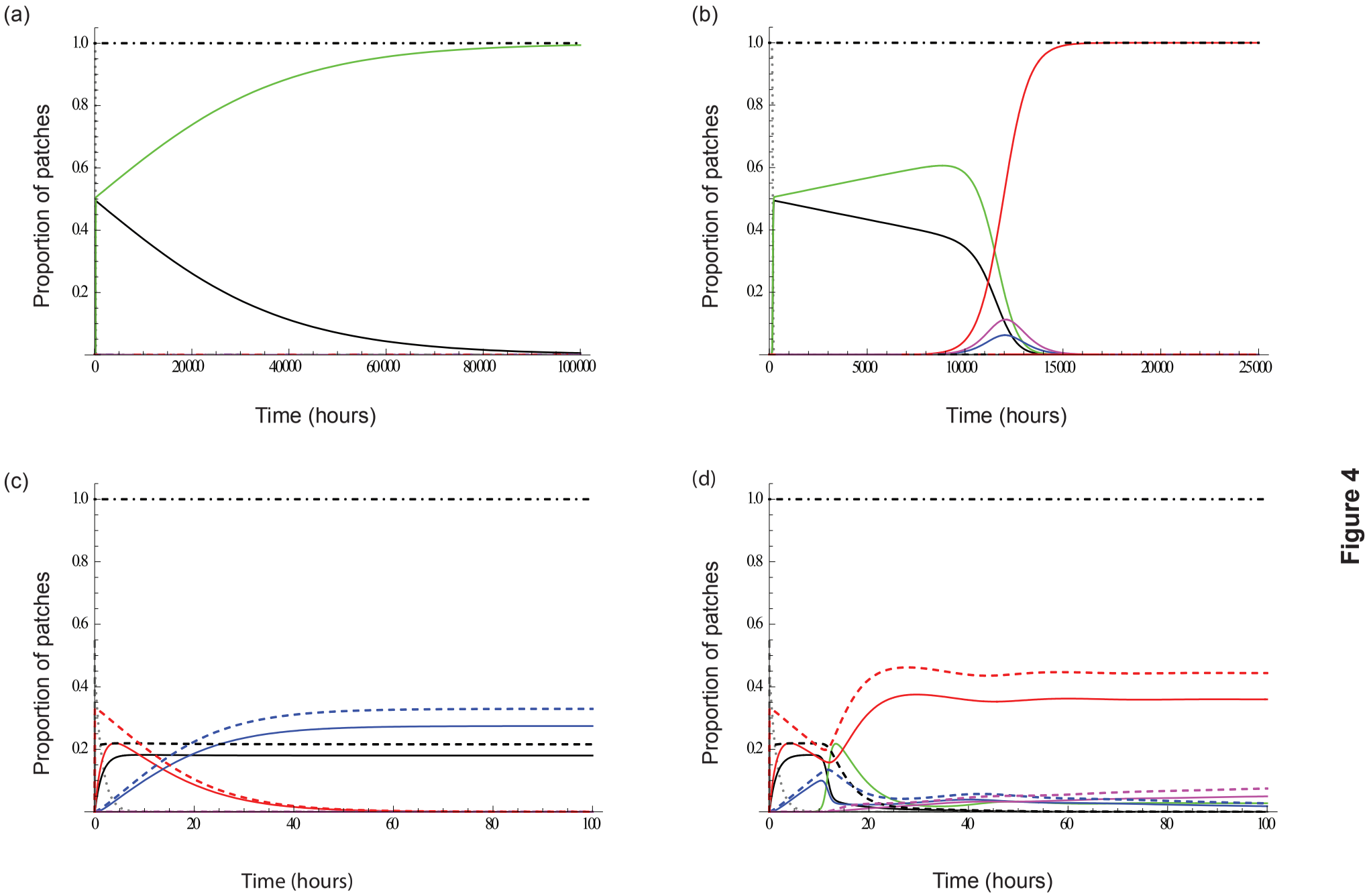

(a)
Proportion of patches
1.0
0.8
0.6
0.4
0.2
0
20000
40000
60000
80000
100000
Time (hours)
(b)
Proportion of patches
1.0
0.8
0.6
0.4
0.2
0
5000
10000
15000
20000
25000
Time (hours)
(c)
Proportion of patches
1.0
0.8
0.6
0.4
0.2
0
20
40
60
80
100
Time (hours)
(d)
Proportion of patches
1.0
0.8
0.6
0.4
0.2
0
20
40
60
80
100
Time (hours)

Figure 4

7. Conclusions

It is widely recognized that bacteria display a diverse range of social traits, from all regions of the social spectrum. Such traits are exchanged between cells not only through vertical transmission from parent to offspring, but also horizontally between different cells. Plasmids are ubiquitous among bacteria and act as vectors for horizontal gene transfer, frequently carrying social traits. In this thesis I have illustrated how plasmid based traits may help their bacterial host in terms of both cooperative (Chapters 2-3) and anti-competitor interactions (Chapter 4) as well as exploring plasmid-transferred resistance to antibiotics (Chapter 6), and conflict between the host and the social plasmid may evolve and be resolved (Chapter 5).

Transmission via plasmids provides three possible advantages for potentially social traits. Firstly, infection by a plasmid forces a cell to carry particular genes, thereby allowing costly genes to spread. This ability to spread infectiously and reprogram the functionality of host cells may even have potential for use in new medical intervention “Trojan horse” strategies. Secondly, the spread of plasmids can increase local relatedness by increasing the numbers of carriers of social genes within an interacting population. Social genes carried on plasmids can influence their social environment to maximize their ability to interact with other carriers of the same social trait. As increased expression of a social gene allows higher levels of public goods, potentially increasing cooperation and productivity, as well as compensating for the low expression of many horizontally acquired traits, a third advantage to plasmid carriage comes from the fact that plasmids are carried in multiple copies thus allowing increased expression of the genes they carry. These factors are illustrated in Figure 3.

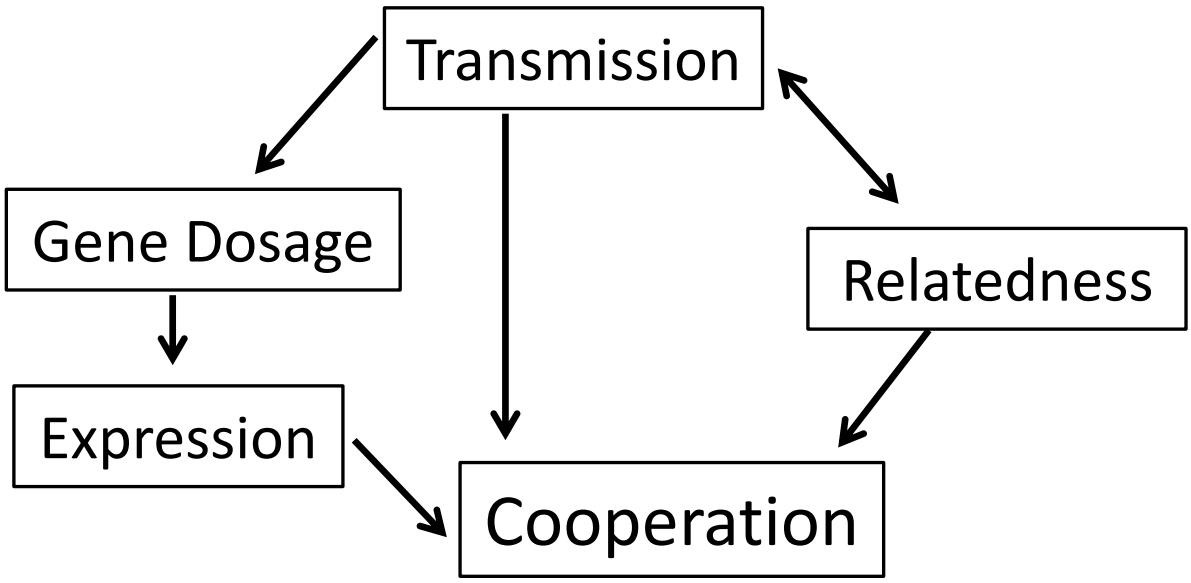


Figure 1. Horizontal gene transfer and the evolution of bacterial cooperation. Transmission spreads the gene itself as well as altering the surrounding population structure. Transfer increases relatedness when cooperation is rare but once it becomes common this effect declines. Transmission via plasmids results in cells carrying multiple copies of the cooperative gene as the plasmid replicates within the host. This increase in gene dosage may lead to increased expression of cooperative traits.

This thesis provides evidence that horizontal gene transfer is not just advantageous for cooperative traits. I also explore the mutually beneficial interaction between plasmids and genes for bacteriocin production whereby plasmids enable bacteriocin producers to spread and bacteriocins maintain plasmids in host cells by damaging cells which lose the plasmid. I examine horizontal transmission of a non-social trait in the form of antibiotic resistance. Plasmid carried resistance outcompetes a chromosomal variant demonstrating that HGT is also advantageous for individually-beneficial traits.

However, there is a darker side to HGT, as conflict and competition may occur between chromosomal and plasmid genes. For instance, in chapter six I find that chromosomally based resistance genes often lose out to plasmid-based equivalents, though not at the expense of the pathogen genome (as resistance is still maintained). This illustrates that horizontal transfer can promote competition between genes. I also explore conflict between a horizontally transferred gene and its host genome as a whole where the host genome can combat the spread of plasmid-based cooperative traits and thereby avoid cooperative behaviour when it is not favourable for the cell.

Overall it therefore appears that the effects of horizontally transmitted genes go beyond the simple concept of "infecting" a cell with a certain gene. In each case where a gene is spread on a plasmid there is likely to be suite of potential interactions: between the plasmid and the gene it carries; between the plasmid and the host cell; or between the horizontally transmitted gene and the host genome (or individual host genes). This thesis merely scratches the surface on a variety of these themes but it demonstrates that there is much going on underneath.

The myriad of genes carried via plasmids, their ubiquity in nature and the ease of which genes can move from chromosome to plasmid and vice versa means that an understanding of the dynamics of these vectors of HGT and realisation of the fluidity of the bacterial gene pool is key to an broader understanding of the evolution of bacteria, and particularly bacterial social evolution.

8 Appendices

Chapter 2 Appendix A: Supplementary methods for model construction

We begin by defining p_{ij} as an indicator variable taking the value one if founder *i* in patch *j* carries the plasmid and zero otherwise. We denote this quantity, when measured after the transmission stage of the lifecycle, as p_{ij}^{t} (where the subscript *t* indicates it is measured after transmission). As p_{ij} depends on which founder is sampled and p_{ij}^{t} depends on founder's descendants are sampled, both p_{ij} and p_{ij}^{t} are random variables. The fitness of an individual will depend on two factors; firstly, whether it carries the plasmid or not after the transmission stage; and secondly, the number of plasmid carriers, after transmission, in that individual's patch (*j*). Therefore we define p_j^t as $p_j^{t} = \sum_i p_{ij}^{t} / N$ which denotes the average frequency of the plasmid, after transmission, in patch *j*. As p_j^t depends on the random variable p_{ij}^{t}, p_j^t is also a random variable.

Because of our life cycle assumptions, where plasmid transmission comes before selection, the fitness of individual *i* in patch *j* (expected number of founders produced by a founder), w_{ij}, refers to individual fitness after transmission. This means that w_{ij} depends on both p_{ij}^{t} and p_j^t, i.e. $w_{ij} = w_{ij}\left(p_{ij}^{t}, p_j^{t}\right)$ and therefore w_{ij} is also a random variable.

We use a standard population genetical approach, and derive our model from the Price Equation (Price 1970; Price 1972) in order to evaluate the change in the average frequency, *p*, of the plasmid in the population (as described formally below). The Price Equation stipulates that the change in the average frequency of the plasmid in the population, Δp, over one generation can be written as:

$$\underbrace{w\Delta p}_{\text{Change in gene frequency}} = \underbrace{\text{Cov}[w_{ij}, p_{ij}]}_{\text{Selection}} + \underbrace{\text{E}[w_{ij}\Delta p_{ij}]}_{\text{Transmission}} \qquad [A1]$$

where Δp_{ij} is the change in an individual's status (plasmid carrier or plasmid free) within a generation; and *w* and *p* are, respectively, the average fitness and average frequency of the plasmid across the whole population. As in previous work (Grafen 1985; Grafen 2008; Taylor 1990; Taylor 1996), the expectation $\text{E}[\cdot]$ denotes an average of a random variable over all individuals in the population; that is, the sum over all individuals within a patch (*i*) summed over all patches (*j*) and divided by the total number of individuals in the population. Formally, the average plasmid frequency in the population is $p = \text{E}[p_{ij}] = \lim_{n\to\infty}\left[\sum_{ij} p_{ij}/(Nn)\right]$ where *n* is the number of patches in the population (assumed infinite) and the average population fitness is $w = \text{E}[w_{ij}] = \lim_{n\to\infty}\left[\sum_{ij} w_{ij}/(Nn)\right]$. We use the covariance $\text{Cov}[\cdot,\cdot]$ to denote the average over all individuals of the product of two quantities minus the product of the averages; for instance for the covariance in equation (A1), we have $\text{Cov}[w_{ij}, p_{ij}] = \text{E}[w_{ij}\,p_{ij}] - \text{E}[p_{ij}]\text{E}[w_{ij}]$.

In equation (A1) we see the change in the average frequency of the plasmid, Δp, depends on the covariance between the plasmid carriage and fitness (which gives the change in the character caused by differential reproductive success i.e. change due to selection) and also on the fitness weighted expectation of the change in character value (the change in character values between ancestor and descendent i.e. change due to plasmid transmission – (Frank 1998)). Writing the change of plasmid frequency in the form of the Price equation allows us

to explicitly partition the effects of selection, through the covariance term, and horizontal gene transfer, through the transmission term.

As $p_{ij} = p_{ij}^{t} - \Delta p_{ij}$, we can rewrite the Price Equation as:

$$w\Delta p = \mathrm{Cov}[w_{ij}, p_{ij}^{\ t}] - \mathrm{Cov}[w_{ij}, \Delta p_{ij}] + \mathrm{E}[w_{ij}\Delta p_{ij}].$$

We can then expand $\mathrm{Cov}[w_{ij}, \Delta p_{ij}]$ in terms of expectations to give:

$$w\Delta p = \mathrm{Cov}[w_{ij}, p_{ij}^{\ t}] - (\mathrm{E}[w_{ij}, \Delta p_{ij}] - \mathrm{E}[w_{ij}]\mathrm{E}[\Delta p_{ij}]) + \mathrm{E}[w_{ij}\Delta p_{ij}].$$

As $w = \mathrm{E}[w_{ij}]$ we can now express equation (A1) in terms of $p_{ij}^{\ t}$ as:

$$\Delta p = \frac{1}{w}\mathrm{Cov}[w_{ij}, p_{ij}^{\ t}] + \mathrm{E}[\Delta p_{ij}] \qquad [A2]$$

This is the expression used in the main text to calculate the change in the average frequency, *p*, of the plasmid in the population (equation (1)).

Chapter 2 Appendix B: Supplementary information and associated supplementary tables

Calculating the selection term $\left(\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}]\right)$:

As $\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] = \mathrm{E}[w_{ij} p_{ij}^{\,t}] - \mathrm{E}[w_{ij}]\mathrm{E}[p_{ij}^{\,t}]$, we can calculate $\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}]$ can be calculated using the fitness term in the main text as:

$$\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] = B(\mathrm{E}[p_{ij}^{\,t} p_j^{\,t}] - p^{t^2}) - C\mathrm{Var}[p^t]. \qquad \text{[B1]}$$

In the above expression, $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$ is the average over all patches of the square of the average plasmid frequency in each patch, which gives the probability that two randomly sampled individuals from a randomly sampled patch in the population will carry the focal allele (that is, the plasmid). $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$ is calculated below (equation (B6)). $\mathrm{Var}[p^t]$ describes the variance in plasmid carriage across individuals and is calculated as

$$\mathrm{Var}[p^t] = \mathrm{E}[p_{ij}^{\,t} p_{ij}^{\,t}] = \left(p_{ij} + (1 - p_{ij})\beta p_j\right)^2 = p_{ij} + (1 - p_{ij})\beta^2 p_j^{\,2}. \qquad \text{[B2]}$$

This allows us to calculate equation (4) in the main text.

Whole-group relatedness

R refers to whole group relatedness, measured after transmission, calculated as

$$R = \frac{\mathrm{E}[p_j^{\,t} p_j^{\,t}] - p^{t\,2}}{\mathrm{Var}[p^t]}, \qquad \text{[B3]}$$

which is the regression of the plasmid frequency after transmission in a patch on the frequency of the plasmid in a focal strain (Frank 1998). We evaluate $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$, in terms of the model's parameters below (equation (B5)). This, together with equation (2) in the main text, allows us to calculate *R* (equation (B6) below).

Within-patch pair identity

The expectation $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$ is described by

$$\mathrm{E}[p_{ij}^{\,t} p_j^{\,t}] = \lim_{n\to\infty}\left[\sum_{ij}\frac{p_{ij}^{\,t} p_j^{\,t}}{nN}\right] = \lim_{n\to\infty}\left[\sum_j \frac{p_j^{\,t}}{n}\sum_i \frac{p_{ij}^{\,t}}{N}\right] = \lim_{n\to\infty}\left[\sum_j \frac{p_j^{\,t} p_j^{\,t}}{n}\right] = \mathrm{E}[p_j^{\,t} p_j^{\,t}] \ .$$

It is the average over all patches of the square of the average plasmid frequency in each patch. It gives the probability that two randomly sampled individuals from a randomly sampled patch will carry the plasmid. We now expand this probability in terms of conditional probabilities as:

$$\mathrm{E}[p_j^{\,t} p_j^{\,t}] = \sum_{i\in s}\left(t_{11\leftarrow i} x_i\right), \qquad \text{(B4)}$$

where $t_{11\leftarrow i}$ is the probability that two plasmid-carrying individuals, randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of origin *i* before transmission (state of the cell before transmission), x_i is the probability of occurrence of the state of origin *i* and $S \in \{1{,}11{,}0{,}00{,}10\}$ is the set of states of origin. The index *i*

represents the original state of the two cells, randomly sampled from the population, before transmission: *i*=1 refers to the case where both have the plasmid (and share the same founder cell), *i*=11 refers to the case where both have the plasmid (but come from different founder cells), *i*=0 refers to the case where neither have the plasmid (and share the same founder cell), *i*=00 refers to the case where neither have the plasmid (but come from different founder cells) and *i*=10 refers to the case where one has the plasmid and the other doesn't (and hence they both come from different founder cells). We now evaluate explicitly the probabilities going into equation (B4).

State of origin probabilities

For the above system we calculate x_i for all *i* (where $i \in S$). The probability that the two individuals sampled both descend from the same plasmid-carrying founder strain is given by:

$$x_1 = \frac{1}{N} p .$$

Where *N* is the number of founder strains and *p* is the frequency of the plasmid in the population. The probability that the two individuals sampled descend from two separate plasmid-carrying founder strains is:

$$x_{11} = \frac{N-1}{N} p^2 .$$

The probability that the two individuals sampled both descend from the same plasmid-free founder strain is given by:

$$x_0 = \frac{1}{N}(1-p)$$

The probability that the two individuals sampled descend from two separate plasmid-free founder strains is given by:

$$x_{00} = \frac{N-1}{N}(1-p)^2$$

Finally, the probability that, of the two individuals sampled, one descends from a plasmid-carrying strain and the other from a plasmid-free strain is given by:

$$x_{10} = 2\frac{N-1}{N}p(1-p).$$

All of these probabilities are summarized in table B1.

Transition probabilities

The transition probabilities are summarized in table B2 and are as follows:

1. *Transition probability,* $t_{11\leftarrow 1}$

The first transition probability, $t_{11\leftarrow 1}$, gives the probability that two randomly sampled individuals which descend from the same plasmid-carrying strain, carry the plasmid after transmission. As both individuals already carry the plasmid this occurs with probability 1, giving $t_{11\leftarrow 1} = 1.$

2. Transition probability $t_{11\leftarrow 11}$

The second transition probability, $t_{11\leftarrow 11}$, describes the probability that two randomly sampled individuals descending from separate plasmid-carrying strains, carry the plasmid after transmission. This also occurs with probability 1, giving $t_{11\leftarrow 11} = 1.$

3. Transition probability $t_{11\leftarrow 0}$

The transition probability, $t_{11\leftarrow 0}$, gives the probability that two randomly sampled individuals, which descend from the same plasmid-free strain, carry the plasmid after transmission. Both of the individuals sampled may be infected upon contact with a plasmid-carrying individual at the transmission stage with probability β^2. All such plasmid-carrying strains are different to the ancestral strain of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which descend from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-1}{N}p\right)$. Alternatively the sampled individuals may infected by plasmid carrying cells which descend from two separate plasmid-carrying strains, this occurs with probability $\left(\frac{N-1}{N}\frac{N-2}{N}p^2\right)$. Thus, the overall transition probability is given by:

$$t_{11\leftarrow 0} = \beta^2\left(\frac{1}{N}\frac{N-1}{N}p + \frac{N-1}{N}\frac{N-2}{N}p^2\right).$$

The β^2 is due to the fact that both cells must have been infected with a plasmid (which occurs with a probability β.

4. Transition probability $t_{11\leftarrow 00}$

The transition probability, $t_{11\leftarrow 00}$, describes the probability that two randomly sampled individuals descending from separate plasmid-free strains, carry the plasmid after transmission. Both may be infected at the transmission stage upon contact with plasmid-carrying individuals with probability β^2. As above, all such plasmid carrying strains are different from the ancestral strains of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which come

from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-2}{N}p\right)$.

Alternatively the sampled individuals may be infected by plasmid-carrying cells from two separate plasmid carrying strains, this occurs with probability $\left(\frac{N-2}{N}\frac{N-3}{N}p^2\right)$. From these the overall conditional probability is given by:

$$t_{11\leftarrow 00} = \beta^2\left(\frac{1}{N}\frac{N-2}{N}p + \frac{N-2}{N}\frac{N-3}{N}p^2\right).$$

5. Transition probability $t_{11\leftarrow 10}$

The transition probability, $t_{11\leftarrow 10}$, describes the probability that two randomly sampled individuals, one descending from a plasmid-free strain and the other from a plasmid-carrying strain, carry the plasmid after transmission. Upon contact with a plasmid-carrier the uninfected sampled individual acquires a plasmid with probability β. It may acquire a plasmid either from a plasmid-carrying cell from the same strain as the sampled infected individual, with probability $\left(\frac{1}{N}\right)$ or from a plasmid-carrying cell from a strain different to both of the ancestral strains of the sampled individuals with probability $\left(\frac{N-2}{N}p_1\right)$. The sampled plasmid-carrying individual is not affected by transmission. From these probabilities, the overall conditional probability for transmission is:

$$t_{1\,1\leftarrow 1\,0}\beta\left(\frac{1}{N} + \frac{N-2}{N}p\right)$$

As only one cell is infected with the plasmid (as the other cell already carries the plasmid), β only appears once in the above equation.

Pair probabilities

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables B1 and B2) and substituting into equation (B4), which gives:

$$\begin{aligned}\mathrm{E}[p_j^{\,\mathrm{t}}\,p_j^{\,\mathrm{t}}] = {} & \frac{1}{N}\,p \\ & + \frac{N-1}{N}\,p^2 \\ & + \frac{1}{N}(1-p)\beta^2\left(\frac{1}{N}\frac{N-1}{N}\,p + \frac{N-1}{N}\frac{N-2}{N}\,p^2\right) \\ & + \frac{N-1}{N}(1-p)^2\beta^2\left(\frac{1}{N}\frac{N-2}{N}\,p + \frac{N-2}{N}\frac{N-3}{N}\,p^2\right) \\ & + 2\frac{N-1}{N}\,p(1-p)\beta\left(\frac{1}{N} + \frac{N-2}{N}\,p\right).\end{aligned} \qquad \text{[B5]}$$

Equation (B5) and equation (2) from the main text are used to calculated relatedness, *R*, equation (B3) in the main text giving, when simplified:

$$R = \frac{N^2\left(1+\beta-2p\beta\right)^2 - 2N\beta\left(1+\beta\text{-}p\left(2-5\left(1-p\right)\beta\right)\right) + \left(1-6\left(1-p\right)p\right)\beta^2}{N\left(N^2-2(N-1)Np\beta+(N-1)\left(1+p(p(N-1)-1)\right)\beta^2\right)}. \qquad \text{[B6]}$$

Supplementary tables for Chapter 2 Appendix B

Table B1. The calculation of x_i.

x_i	Probability
x_1	$\frac{1}{N}p$
x_{11}	$\frac{N-1}{N}p^2$
x_0	$\frac{1}{N}(1-p)$
x_{00}	$\frac{N-1}{N}(1-p)^2$
x_{10}	$2\frac{N-1}{N}p(1-p)$

Table B2. The calculation of $t_{11\leftarrow i}$.

$t_{11\leftarrow i}$	Probability
$t_{11\leftarrow 1}$	1
$t_{11\leftarrow 11}$	1
$t_{11\leftarrow 0}$	$\beta^2\left(\frac{1}{N}\frac{N-1}{N}p+\frac{N-1}{N}\frac{N-2}{N}p^2\right)$
$t_{11\leftarrow 00}$	$\beta^2\left(\frac{1}{N}\frac{N-2}{N}p+\frac{N-2}{N}\frac{N-3}{N}p^2\right)$
$t_{11\leftarrow 10}$	$\beta\left(\frac{1}{N}+\frac{N-2}{N}p\right)$

Chapter 3 Appendix A: Supplementary methods for construction of gene duplication model

We begin by defining p_{ij} as an indicator variable taking the value one if founder *i* in patch *j* carries the focal allele (a chromosomally-encoded duplicated gene or a plasmid that encodes the second copy of the gene) and zero otherwise. Because the plasmid can be transferred horizontally, we must incorporate a transmission stage in our lifecycle as detailed in the main text. We denote the indicator variable, when measured after the transmission stage of the lifecycle, as p_{ij}^{t} (where the subscript *t* indicates it is measured after transmission). Because p_{ij} depends on which founder is sampled, and p_{ij}^{t} depends on which of the founder's descendants are sampled, both p_{ij} and p_{ij}^{t} are random variables. The fitness of an individual will depend on two factors; firstly, whether it carries the focal allele or not after the transmission stage; and secondly, the number of carriers of the focal allele, after transmission, in that individual's patch (*j*). Therefore we define p_j^t as $p_j^{t} = \sum_i p_{ij}^{t} / N$, which denotes the average frequency of the focal allele after transmission, in patch *j*. Because p_j^t depends on the random variable p_{ij}^{t}, p_j^t is also a random variable.

In the life cycle we assume (see main text), plasmid transmission comes before selection. Therefore, the fitness of individual *i* in patch *j* (expected number of founders produced by a founder), w_{ij}, refers to individual fitness after transmission. This means that w_{ij} depends on both p_{ij}^{t} and p_j^t , i.e. $w_{ij} = w_{ij}\left(p_{ij}^{t}, p_j^{t}\right)$. In consequence, w_{ij} is also a random variable.

We use a standard population genetic approach, and derive our model from the Price Equation (Price 1970; Price 1972) in order to evaluate the change in the average frequency, *p*, of the

focal allele in the population (as described formally below). The Price Equation stipulates that the change in the average frequency of the focal allele, *p*, in the population, Δp, over one generation can be written as:

$$\underbrace{w\Delta p}_{\text{Change in gene frequency}} = \underbrace{\text{Cov}[w_{ij}, p_{ij}]}_{\text{Selection}} + \underbrace{\text{E}[w_{ij}\Delta p_{ij}]}_{\text{Transmission}} \qquad \text{[A1]}$$

where Δp_{ij} is the change in an individual's status (carrier of the second copy of the gene, either on the chromosome or on a plasmid) within a generation; and *w* and *p* are, respectively, the average fitness and average frequency of the focal allele across the whole population. As in previous work (Grafen 1985; Grafen 2008; Taylor 1990; Taylor 1996), the expectation $\text{E}[\cdot]$ denotes an average of a random variable over all individuals in the population; that is, the sum over all individuals within a patch (*i*) summed over all patches (*j*) and divided by the total number of individuals in the population. Formally, the average frequency of *p* in the population is $p = \text{E}[p_{ij}] = \lim_{n\to\infty}\left[\sum_{ij} p_{ij}/(Nn)\right]$ where *n* is the number of patches in the population (assumed to be infinite) and the average population fitness is $w = \text{E}[w_{ij}] = \lim_{n\to\infty}\left[\sum_{ij} w_{ij}/(Nn)\right]$. We use the covariance $\text{Cov}[\cdot,\cdot]$ to denote the average over all individuals of the product of two quantities minus the product of the averages; for instance for the covariance in equation (A1), we have $\text{Cov}[w_{ij}, p_{ij}] = \text{E}[w_{ij}p_{ij}] - \text{E}[p_{ij}]\text{E}[w_{ij}]$.

Because $p_{ij} = p_{ij}^{\text{t}} - \Delta p_{ij}$, we can rewrite the Price Equation as

$$w\Delta p = \text{Cov}[w_{ij}, p_{ij}^{\text{t}}] - \text{Cov}[w_{ij}, \Delta p_{ij}] + \text{E}[w_{ij}\Delta p_{ij}].$$

We can then expand $\mathrm{Cov}[w_{ij}, \Delta p_{ij}]$ in terms of expectations to give:

$$w\Delta p = \mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] - (\mathrm{E}[w_{ij}, \Delta p_{ij}] - \mathrm{E}[w_{ij}]\mathrm{E}[\Delta p_{ij}]) + \mathrm{E}[w_{ij}\Delta p_{ij}].$$

Because $w = \mathrm{E}[w_{ij}]$ we can now express equation (A1) in terms of $p_{ij}^{\,t}$ as

$$\Delta p = \frac{1}{w}\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] + \mathrm{E}[\Delta p_{ij}] \qquad [A2]$$

This is the expression we use to calculate the change in the average frequency, *p*, of the focal allele in the population. We next explain how we calculate each component of the right hand side of this equation.

Transmission $(\mathrm{E}[\Delta p_{ij}])$

The change in frequency due to transmission, $\mathrm{E}[\Delta p_{ij}]$, is calculated using the life cycle described in the main text. Because $\mathrm{E}[\Delta p_{ij}] = \mathrm{E}[p_{ij}^{\,t}] - \mathrm{E}[p_{ij}]$ and $\mathrm{E}[p_{ij}] = p$ we need only calculate $\mathrm{E}[p_{ij}^{\,t}]$ which, when the second copy of the gene is carried on a plasmid, is given by:

$$\mathrm{E}[p_{ij}^{\,t}] = p^t = p + (1-p)\frac{N-1}{N}\beta p, \qquad [A3]$$

This equation is composed of the sum of the average population frequency of those individuals who originally carried plasmid (*p*), plus those non-carriers who were infected with the plasmid (1 - *p*) at the transmission stage. These plasmid-free individuals are infected with probability β by plasmid-carrying individuals that are descended from a strain different from their own, which occurs with probability $((N-1)/N)p$, where *N* is the number of founding strains in a patch. The sum of these terms gives the average frequency of the plasmid in the population after transmission (p^t). It follows that $\mathrm{E}[\Delta p_{ij}] = p + (1-p)((N-1)/N)\beta p - p.$, and we see that the change in frequency after one generation depends on the variance in the population $(p(1-p))$, and a transfer coefficient based on the number of strains and the probability of transfer $((N-1)/N)\beta$.

We note that for chromosomally based gene duplications there is no horizontal gene transfer and thus

$\mathrm{E}[p_{ij}{}^t] = p^t = p$ and $\mathrm{E}[\Delta p_{ij}] = 0$.

Fitness functions

We consider a population which is already producing public goods, where *p* refers to a duplicate gene (encoded on a plasmid or the chromosome) that increases this production. We calculate the fitness of an individual, *i*, carrying the duplicated gene, in patch *j*, as

$$w_{ij} = 1 - (1 - p_{ij}{}^t)c - p_{ij}{}^t cy + (1 - p_j{}^t)b + p_j{}^t bx.$$

The cost of producing the public good to the producer individual (*i*) is represented by *c*. The variable *b* represents the benefit of producer behavior (shared by all individuals within the same patch *j*). This is the baseline level of public goods production in the population. Cells

containing the duplicated gene produce an additional amount of public good *bx* at an additional cost *cy* (as described in the main text). Therefore, mean fitness across the whole population, after transmission and fitness interaction, can be calculated as

$$w = 1 - (1 - p^{t})c - p^{t}cy + (1 - p^{t})b + p^{t}bx.$$

Calculating the selection term $\left(\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}]\right)$

Because $\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] = \mathrm{E}[w_{ij}\, p_{ij}^{\,t}] - \mathrm{E}[w_{ij}]\mathrm{E}[p_{ij}^{\,t}]$, we can calculate $\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}]$ using the fitness term in the main text as:

$$\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] = bx(\mathrm{E}[p_{ij}^{\,t} p_{j}^{\,t}] - p^{t^2}) - cy\mathrm{Var}[p^{t}]. \qquad \text{[A4]}$$

Using our selection and transmission terms and equation (A1) we can describe the full model for the change in frequency of the focal allele as follows

$$\Delta p = bx(\mathrm{E}[p_{ij}^{\,t} p_{j}^{\,t}] - p^{t^2}) - cy\mathrm{Var}[p^{t}] + (1 - p)\frac{N-1}{N}\beta p,$$

in the case of a plasmid encoded gene. In the case of a chromosomal duplication the full model is as follows:

$$\Delta p = bx(\mathrm{E}[p_{ij}^{\,t} p_{j}^{\,t}] - p^{t^2}) - cy\mathrm{Var}[p^{t}].$$

In the above expression, $\mathrm{E}[p_{j}^{\,t} p_{j}^{\,t}]$ is the average, over all patches, of the square of the average frequency of the focal allele (i.e. plasmid or chromosomally encoded duplicate gene)

in each patch, which gives the probability that two randomly sampled individuals from a randomly sampled patch in the population will carry the focal allele. $\mathrm{E}[p_j^{\mathrm{t}} p_j^{\mathrm{t}}]$ is calculated below (equation (A6)). $\mathrm{Var}[p^{\mathrm{t}}]$ describes the variance in gene duplication carriage across individuals and is calculated as

$$\mathrm{Var}[p^{\mathrm{t}}] = \mathrm{E}[p_{ij}^{\mathrm{t}} p_{ij}^{\mathrm{t}}] = \left(p_{ij} + (1-p_{ij})\beta p_j\right)^2 = p_{ij} + (1-p_{ij})\beta^2 {p_j}^2. \qquad [A5]$$

Within-patch pair identity

The expectation $\mathrm{E}[p_j^{\mathrm{t}} p_j^{\mathrm{t}}]$ is described by

$$\mathrm{E}[{p_{ij}}^{\mathrm{t}} {p_j}^{\mathrm{t}}] = \lim_{n\to\infty}\left[\sum_{ij} \frac{{p_{ij}}^{\mathrm{t}} {p_j}^{\mathrm{t}}}{nN}\right] = \lim_{n\to\infty}\left[\sum_{j} \frac{{p_j}^{\mathrm{t}}}{n} \sum_{i} \frac{{p_{ij}}^{\mathrm{t}}}{N}\right] = \lim_{n\to\infty}\left[\sum_{j} \frac{{p_j}^{\mathrm{t}} {p_j}^{\mathrm{t}}}{n}\right] = \mathrm{E}[{p_j}^{\mathrm{t}} {p_j}^{\mathrm{t}}] \ .$$

It is the average over all patches of the square of the average frequency of the duplicate gene in each patch, and gives the probability that two randomly sampled individuals from a randomly sampled patch will carry the duplicate gene. We now expand this probability in terms of conditional probabilities as

$$\mathrm{E}[p_j^{\mathrm{t}} p_j^{\mathrm{t}}] = \sum_{i\in s} \left(t_{11\leftarrow i} x_i\right) , \qquad (A6)$$

where $t_{11\leftarrow i}$ is the probability that two duplicate gene-carrying individuals, randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of

origin *i* before transmission (state of the cell before transmission), x_i is the probability of occurrence of the state of origin *i* and $S \in \{1,11,0,00,10\}$ is the set of states of origin. The index *i* represents the original state of the two cells, randomly sampled from the population, before transmission: *i*=1 refers to the case where both have the duplicate gene (and share the same founder cell), *i*=11 refers to the case where both have the duplicate gene (but come from different founder cells), *i*=0 refers to the case where neither have the duplicate gene (and share the same founder cell), *i*=00 refers to the case where neither have the duplicate gene (but come from different founder cells) and *i*=10 refers to the case where one has the duplicate gene and the other doesn't (and hence they both come from different founder cells). We now evaluate explicitly the probabilities going into equation (A6).

State of origin probabilities

For the above system we calculate x_i for all *i* (where $i \in S$). The probability that the two individuals sampled both descend from the same duplicate gene -carrying founder strain is given by:

$$x_1 = \frac{1}{N} p .$$

Where *N* is the number of founder strains and *p* is the frequency of the duplicate gene in the population. The probability that the two individuals sampled descend from two separate duplicate gene -carrying founder strains is:

$$x_{11} = \frac{N-1}{N} p^2 .$$

The probability that the two individuals sampled both descend from the same founder strain (which does not carry the duplicate gene) is given by:

$$x_0 = \frac{1}{N}(1-p)$$

The probability that the two individuals sampled descend from two separate founder strains (neither of which carry the duplicate gene) is given by:

$$x_{00} = \frac{N-1}{N}(1-p)^2$$

Finally, the probability that, of the two individuals sampled, one descends from a strain carrying the duplicate gene and the other from a strain which does not is given by:

$$x_{10} = 2\frac{N-1}{N}p(1-p).$$

All of these probabilities are summarized in table A1.

Transition probabilities

The transition probabilities are summarized in table A2 and are as follows:

1. *Transition probability,* $t_{11\leftarrow 1}$

The first transition probability, $t_{11\leftarrow 1}$, gives the probability that two randomly sampled individuals which descend from the same strain (which carries the duplicate gene), carry the duplicate gene after transmission. Because both individuals already carry the plasmid this occurs with probability 1, giving $t_{11\leftarrow 1} = 1.$

2. Transition probability $t_{11\leftarrow 11}$

The second transition probability, $t_{11\leftarrow 11}$, describes the probability that two randomly sampled individuals descending from separate strains (both of which carry the duplicate gene), carry the duplicate gene after transmission. This also occurs with probability 1, giving $t_{11\leftarrow 11}=1.$

3. Transition probability $t_{11\leftarrow 0}$

The transition probability, $t_{11\leftarrow 0}$, gives the probability that two randomly sampled individuals, which descend from the same strain (which does not carry the duplicate gene), carry the duplicate gene after transmission. This transition can only occur when the duplicate gene is carried on a plasmid as the chromosomal duplicate gene is not spread horizontally. Both of the individuals sampled may be infected upon contact with a plasmid-carrying individual at the transmission stage with probability β^2. All such plasmid-carrying strains are different from the ancestral strain of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which descend from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-1}{N}p\right)$. Alternatively, the sampled individuals may infected by plasmid carrying cells which descend from two separate plasmid-carrying strains. This occurs with probability $\left(\frac{N-1}{N}\frac{N-2}{N}p^2\right)$. Thus, the overall transition probability is given by:

$$t_{11\leftarrow 0}=\beta^2\left(\frac{1}{N}\frac{N-1}{N}p+\frac{N-1}{N}\frac{N-2}{N}p^2\right)$$

The factor β^2 is due to the fact that both cells must have been infected with a plasmid (which occurs with a probability β.

4. Transition probability $t_{11\leftarrow 00}$

The transition probability, $t_{11\leftarrow 00}$, describes the probability that two randomly sampled individuals descending from separate strains, neither of which carry the duplicate gene, carry it after the transmission stage. Again, this transition can only occur when the duplicate gene is carried on a plasmid. Both may be infected at the transmission stage upon contact with plasmid-carrying individuals, which occurs with probability β^2. As above, all such plasmid carrying strains are different from the ancestral strains of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which come from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-2}{N}p\right)$. Alternatively the sampled individuals may be infected by plasmid-carrying cells from two separate plasmid carrying strains, this occurs with probability $\left(\frac{N-2}{N}\frac{N-3}{N}p^2\right)$. From these the overall conditional probability calculates as

$$t_{11\leftarrow 00}=\beta^2\left(\frac{1}{N}\frac{N-2}{N}p+\frac{N-2}{N}\frac{N-3}{N}p^2\right).$$

5. Transition probability $t_{11\leftarrow 10}$

The transition probability $t_{11\leftarrow 10}$, describes the probability that two randomly sampled individuals, one descending from a duplicate-gene-free strain and the other from a duplicate-gene-carrying strain, carry the duplicate gene after transmission. As in the above transition, this transition can only occur when the duplicate gene is carried on a plasmid. Upon contact with a plasmid-carrier the uninfected sampled individual acquires a plasmid with probability β. It may acquire a plasmid either from a plasmid-carrying cell from the same strain as the

sampled infected individual, with probability $\left(\frac{1}{N}\right)$, or from a plasmid-carrying cell from a strain different to both of the ancestral strains of the sampled individuals with probability $\left(\frac{N-2}{N}p_1\right)$. The sampled plasmid-carrying individual is not affected by transmission. From these probabilities, the overall conditional probability for transmission is:

$$t_{11\leftarrow 10}\beta\left(\frac{1}{N}+\frac{N-2}{N}p\right)$$

Because only one cell is infected with the plasmid (the other cell already carries the plasmid), β only appears once in the above equation.

Pair probabilities

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables A1 and A2) and substituting into equation (A6), which gives

$$\begin{aligned}\mathrm{E}[p_j^{\,t}p_j^{\,t}] = {} & \frac{1}{N}p \\ & + \frac{N-1}{N}p^2 \\ & + \frac{1}{N}(1-p)\beta^2\left(\frac{1}{N}\frac{N-1}{N}p+\frac{N-1}{N}\frac{N-2}{N}p^2\right) \\ & + \frac{N-1}{N}(1-p)^2\beta^2\left(\frac{1}{N}\frac{N-2}{N}p+\frac{N-2}{N}\frac{N-3}{N}p^2\right) \\ & + 2\frac{N-1}{N}p(1-p)\beta\left(\frac{1}{N}+\frac{N-2}{N}p\right).\end{aligned} \qquad \text{[A7]}$$

In the case of a chromosomally encoded duplication there is no transfer via β and thus the expression for $\mathrm{E}[p_j^{\mathrm{t}} p_j^{\mathrm{t}}]$ becomes

$$\mathrm{E}[p_j^{\mathrm{t}} p_j^{\mathrm{t}}] = \frac{1}{N} p + \frac{N-1}{N} p^2. \qquad [A8]$$

Chapter 3 Appendix A Supplementary Tables

Table A1. The calculation of x_i.

x_i	Probability
x_1	$\frac{1}{N} p$
x_{11}	$\frac{N-1}{N} p^2$
x_0	$\frac{1}{N}(1-p)$
x_{00}	$\frac{N-1}{N}(1-p)^2$
x_{10}	$2\frac{N-1}{N} p(1-p)$

Table A2. The calculation of $t_{11\leftarrow i}$.

$t_{11\leftarrow i}$	Probability
$t_{11\leftarrow 1}$	1
$t_{11\leftarrow 11}$	1
$t_{11\leftarrow 0}$	$\beta^2\left(\frac{1}{N}\frac{N-1}{N}p+\frac{N-1}{N}\frac{N-2}{N}p^2\right)$
$t_{11\leftarrow 00}$	$\beta^2\left(\frac{1}{N}\frac{N-2}{N}p+\frac{N-2}{N}\frac{N-3}{N}p^2\right)$
$t_{11\leftarrow 10}$	$\beta\left(\frac{1}{N}+\frac{N-2}{N}p\right)$

Chapter 3 Appendix B: Supplementary Information

Chapter 3 Supplementary Tables

Supplementary Table 1. Summary of data set of 696,339 proteins with known and singular localizations. This data is a subset of a total dataset of 1,043,170 proteins which also includes proteins with localisations, classified as unknown and multiple. Analyses were based on this dataset, a summary of which is given below.

Localisation	Number of proteins	Percentage of total (%)
Extracellular	11077	1.59
Cytoplasm	415977	59.74
Cytoplasmic membrane	222823	32.00
Outer membrane	19691	2.83
Periplasm	26771	3.84

Supplementary Table 2. Outgroups

Genus	Outgroup	No. of strains per genus
Acinetobacter	Psychrobacter PRwf-1	8
Bartonella	Sinorhizobium meliloti	4
Brucella	Rhizobium NGR234	10
Buchnera	Candidatus Blochmannia floridanus	6
Burkholderia	Pseudomonas syringae pv_B728a	25
Campylobacter	Wolinella succinogenes	7
Chlamydia	Chlamydophila caviae	6
Chlamydophila	Chlamydia muridarum	6
Chlorobium	Porphyromonas gingivalis W83	6
Coxiella	Pseudomonas aeruginosa	4
Desulfovibrio	Geobacter sulfurreducens	6
Escherichia	Escherichia fergusonii ATCC_35469	36
Francisella	Agrobacterium tumefaciens C58 Cereon	8
Geobacter	Bdellovibrio bacteriovorus	6
Haemophilus	Pasteurella multocida	8
Helicobacter	Wolinella succinogenes	11
Legionella	Coxiella burnetii	6
Methylobacterium	Rhodopseudomonas palustris BisA53	8
Neisseria	Chromobacterium violaceum	7
Prochlorococcus	Synechococcus sp WH8102	12
Pseudomona	Shewanella oneidensis	15
Rhodobacter	Paracoccus denitrificans PD1222	5
Rhodopseudomonas	Bradyrhizobium japonicum	6
Rickettsia	Wolbachia wRi	11
Salmonella	Escherichia coli 536	15
Shewanella	Photobacterium profundum SS9	13
Thermotoga	Aquifex aeolicus	5
Vibrio	Photobacterium profundum SS9	10
Xanthomonas	Xylella fastidiosa	8
Yersinia	Photorhabdus luminescens	13

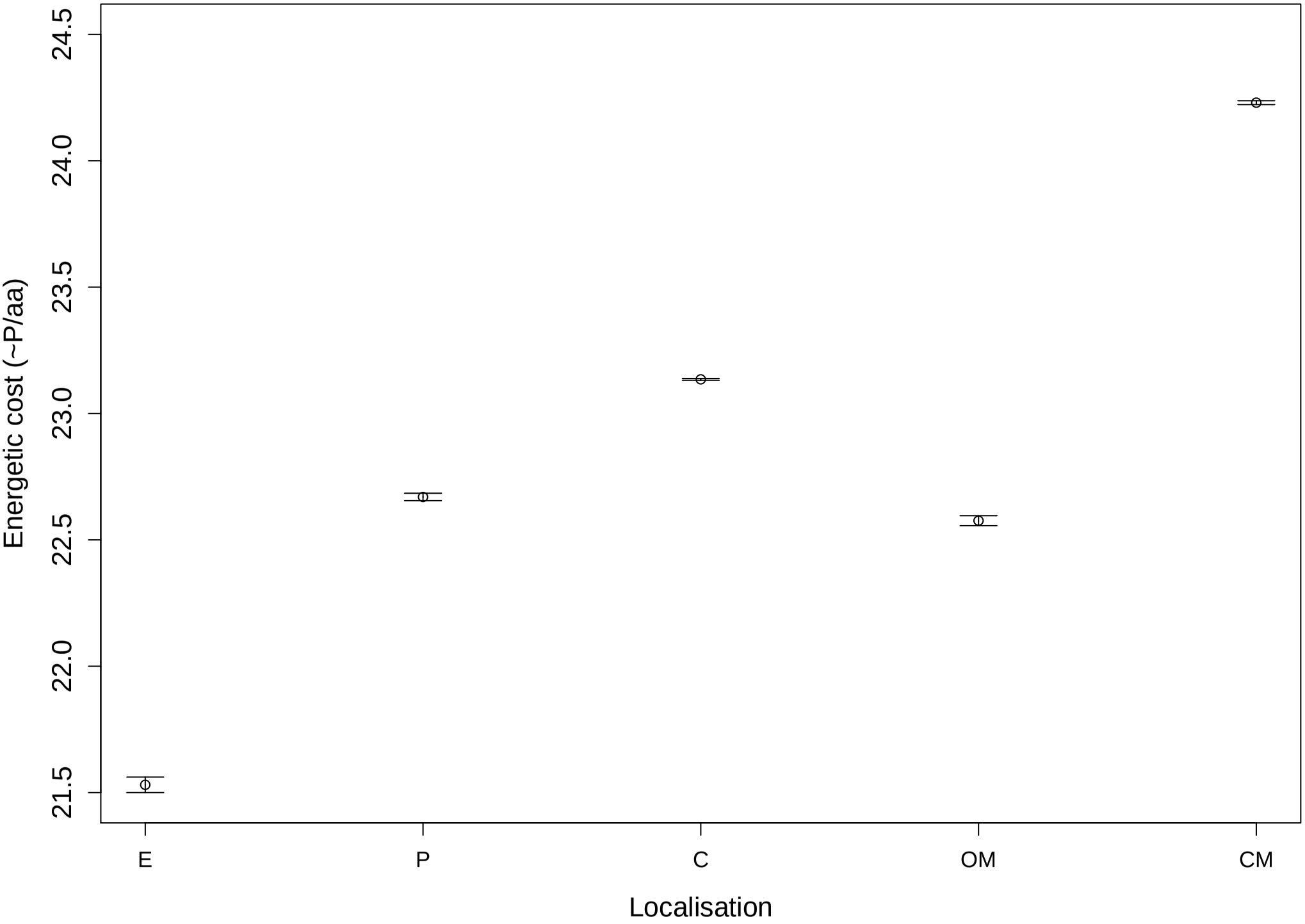
Chapter 3 Supplementary Figure Legends

Supplementary Figure 1. Secreted proteins are less expensive than other proteins.

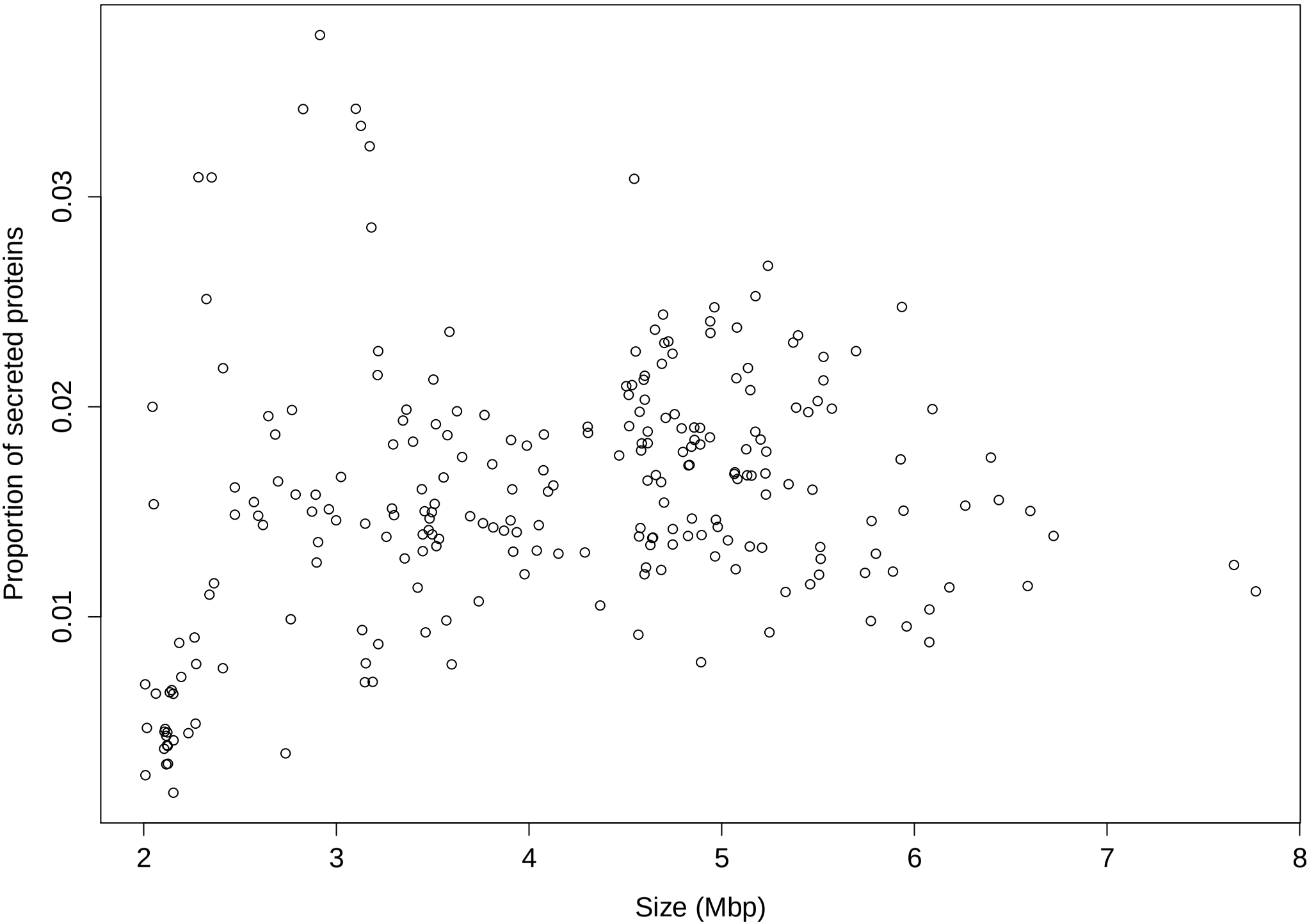
The horizontal axis shows the five protein localisations. The vertical axis shows the energy cost to synthesize each of our study proteins. The cost is estimated as the number of ATP molecules needed for the synthesis of each of the 20 natural amino acids. Points are averages of costs per localisation with 95% confidence intervals. This pattern is reflected in Nogueira et al (2009), although the addition of more data seems to have reduced average costs.

Supplementary Figure 2. There is no negative association between chromosome size and the proportion of secreted proteins encoded thereon. The horizontal axis shows plasmid size in Mega base pairs (Mbp), the vertical axis proportion of genes on a plasmid that encode secreted proteins.

Supplementary Figure 1



Supplementary Figure 2



Chapter 4 Appendix A: Supplementary information for plasmid-carried bacteriocins model

Full model derivation

We can express individual fitness after transmission (with model parameters listed in Table 1 in the main text) as:

$$w_{ij} = 1 - C_1 p_{ij}^{\,t} - (1 - p_{ij}^{\,t}) p_j^{\,t} v.$$

The average fitness of all cells in the population is:

$$w = 1 - C_1 p^t - v p^t (1 - p^t) - v R^t \, \mathrm{Var}[p^t],$$

where R^t refers to whole group relatedness, measured after transmission, calculated as

$$R^t = \frac{\mathrm{E}[p_j^{\,t} p_j^{\,t}] - p^{t\,2}}{\mathrm{Var}[p^t]}, \qquad \text{[A1]}$$

which is the regression of the plasmid frequency after transmission in a patch on the frequency of the plasmid in a focal strain (Frank 1998). $\mathrm{Var}[p^t]$ describes the variance in plasmid carriage across individuals and is calculated as

$$\mathrm{Var}[p^t] = \mathrm{E}[p_{ij}^{\,t} p_{ij}^{\,t}] - \mathrm{E}[p_{ij}^{\,t}]^2, \qquad \text{[A2]}$$

Where $\mathrm{E}[p_{ij}^{\,t} p_{ij}^{\,t}] = \left(p_{ij} + (1 - p_{ij})\beta p_j\right)^2 = \mathrm{E}\left[p_{ij} + (1 - p_{ij})\beta^2 p_j^{\,2}\right]$

$\mathrm{E}[p_j^{\,t} p_j^{\,t}]$ is the probability that two cells in a patch (picked at random after transmission) both carry the plasmid, where:

$$\mathrm{E}[p_{ij}^{\,t} p_j^{\,t}] = \lim_{n\to\infty}\left[\sum_{ij}\frac{p_{ij}^{\,t} p_j^{\,t}}{nN}\right] = \lim_{n\to\infty}\left[\sum_{j}\frac{p_j^{\,t}}{n}\sum_{i}\frac{p_{ij}^{\,t}}{N}\right] = \lim_{n\to\infty}\left[\sum_{j}\frac{p_j^{\,t} p_j^{\,t}}{n}\right] = \mathrm{E}[p_j^{\,t} p_j^{\,t}].$$

$\mathrm{E}[p^t_j p^t_j]$ is derived in a later section of this Appendix (see equation (A4-A5)).

Substituting equations (3-5) into equation (2) in the main text, and simplifying gives the full model for the change in frequency of the plasmid over a single generation:

$$\Delta p = \frac{1}{w}\left(-\mathrm{Var}[p^{\mathrm{t}}]\left(C_1 + vR^t\right) - v\mathrm{E}[p_{ij}^{\,t} p_j^{\,t}]p^t + v\mathrm{E}[p_{ij}^{\,t} p_{ij}^{\,t} p_j^{\,t}]\right) + \left(\frac{N-1}{N}\beta\right)p(1-p)$$

[A3]

Where $\mathrm{E}[p_{ij}^{\,t} p_{ij}^{\,t} p_j^{\,t}]$ is calculated as:

$\mathrm{E}[p_{ij}^{\,t} p_{ij}^{\,t} p_j^{\,t}] = \left(p_{ij} + (1-p_{ij})\beta p_j\right)\left(p_{ij} + (1-p_{ij})\beta p_j\right)\left(p_j + (1-p_j)\beta p_j\right)$. This gives:

$\mathrm{E}[p_{ij}^{\,t} p_{ij}^{\,t} p_j^{\,t}] = \mathrm{E}[p_{ij}p_j] + (1+\beta)+\beta(\beta(\mathrm{E}[p_j p_j p_j] + (\mathrm{E}[p_{ij}p_j p_j p_j] + \mathrm{E}[p_j p_j p_j] - \mathrm{E}[p_j p_j p_j p_j] - \mathrm{E}[p_{ij}p_j p_j])\beta$

$-\mathrm{E}[p_{ij}p_j p_j p_j](1+\beta))$. The moments required for the calculation of $\mathrm{E}[p_{ij}^{\,t} p_{ij}^{\,t} p_j^{\,t}]$ are displayed in Table A1.

Within-patch pair identities $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$

The expectation $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$ is the average over all patches of the square of the average plasmid frequency in each patch, and since the number of patches is infinite it gives the probability that two randomly sampled individuals from a randomly sampled patch will carry the plasmid. We now expand this probability in terms of conditional probabilities as:

$$\mathrm{E}[p_j{}^{\mathrm{t}} p_j{}^{\mathrm{t}}] = \sum_{i \in s} \left(t_{pp \leftarrow i} x_i \right), \tag{A4}$$

where $t_{pp \leftarrow i}$ is the probability that two plasmid-carrying individuals, randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of origin *i* before transmission (state of the cell before transmission), x_i is the probability of occurrence of the state of origin *i* and $S \in \{p, pp, 0, 00, p0\}$ is the set of states of origin. The index *i* represents the original state of the two cells, randomly sampled from the population, before transmission: *i*=*p* refers to the case where both have the plasmid (and share the same founder cell), *i*=*pp* refers to the case where both have the plasmid (but come from different founder cells), *i*=0 refers to the case where neither have the plasmid (and share the same founder cell), *i*=00 refers to the case where neither have the plasmid (but come from different founder cells) and *i*=*p*0 refers to the case where one has the plasmid and the other doesn't (and hence they both come from different founder cells). We now evaluate explicitly the probabilities going into equation (A4).

State of origin probablities

We will now calculate the probability of occurrence of the state of origin *i* of the two descendant individuals. This probability is given by $x_{i,}$ where $i \in S$.

The probability that the two individuals sampled both descend from the same plasmid-carrying founder strain is given by:

$$x_p = \frac{1}{N} p .$$

The probability that the two individuals sampled descend from two separate plasmid-carrying founder strains is:

$$x_{pp} = \frac{N-1}{N} p^2 .$$

The probability that the two individuals sampled both descend from the same plasmid-free, founder strain is given by:

$$x_0 = \frac{1}{N}(1-p)$$

The probability that the two individuals sampled descend from two separate plasmid-free, founder strains is given by:

$$x_{00} = \frac{N-1}{N}(1-p)^2$$

The probability that, of the two individuals sampled, one descends from a plasmid-carrying strain and the other from a plasmid-free strain is given by:

$$x_{p0} = 2\frac{N-1}{N} p(1-p) .$$

All of these probabilities are summarized in Table A2.

Sampling two cells with plasmids (E[$p_j p_j$])

We now calculate the transition probability $t_{pp\leftarrow i}$, the probability that two plasmid-carrying individuals, randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of origin *i* before transmission (state of the cell before transmission). The components of $t_{pp\leftarrow i}$, which are listed in Table A3, are given below.

1. Transition probability, $t_{pp\leftarrow p}$

The first transition probability, $t_{pp\leftarrow p}$, gives the probability that two randomly sampled individuals which descend from the same plasmid-carrying strain, carry the plasmid after transmission. As both individuals already carry the plasmid this occurs with probability 1, giving $t_{pp\leftarrow p}=1.$

2. Transition probability $t_{pp\leftarrow pp}$

The second transition probability, $t_{pp\leftarrow pp}$, describes the probability that two randomly sampled individuals descending from separate plasmid-carrying strains, carry the plasmid after transmission. This also occurs with probability 1, giving $t_{pp\leftarrow pp}=1$.

3. Transition probability $t_{pp\leftarrow 0}$

The transition probability, $t_{pp\leftarrow 0}$, gives the probability that two randomly sampled individuals, which descend from the same plasmid-free strain, carry the plasmid after transmission. Both of the individuals sampled may be infected upon contact with a plasmid-carrying individual at the transmission stage with probability β^2. All such plasmid-carrying strains are different to the ancestral strain of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which descend from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-1}{N}p\right)$. Alternatively the sampled individuals may infected by plasmid-carrying cells which descend from two separate plasmid 1-carrying strains, this occurs with probability $\left(\frac{N-1}{N}\frac{N-2}{N}p^2\right)$.

Thus, the overall transition probability is given by:

$$t_{pp\leftarrow 0} = \beta^2\left(\frac{1}{N}\frac{N-1}{N}p + \frac{N-1}{N}\frac{N-2}{N}p^2\right).$$

The β^2 is due to the fact that both cells must have been infected with a plasmid (which occurs with a probability β.

4. Transition probability $t_{pp\leftarrow 00}$

The transition probability, $t_{pp\leftarrow 00}$, describes the probability that two randomly sampled individuals descending from separate plasmid-free strains, carry the plasmid after transmission. Both may be infected at the transmission stage upon contact with plasmid-carrying individuals at rate β^2. As above, all such plasmid-carrying strains are different from the ancestral strains of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which come from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-2}{N}p\right)$. Alternatively the sampled individuals may be infected by plasmid-carrying cells from two separate plasmid-carrying strains, this occurs with probability $\left(\frac{N-2}{N}\frac{N-3}{N}p^2\right)$. From these the overall conditional probability is given by:

$$t_{pp\leftarrow 00} = \beta^2\left(\frac{1}{N}\frac{N-2}{N}p + \frac{N-2}{N}\frac{N-3}{N}p^2\right).$$

5. Transition probability $t_{pp\leftarrow p0}$

The transition probability, $t_{pp\leftarrow p0}$, describes the probability that two randomly sampled individuals, one descending from a plasmid-free strain and the other from a plasmid-carrying strain, carry the plasmid after transmission. Upon contact with a plasmid carrier the

uninfected sampled individual acquires the plasmid with probability β. It may acquire a copy of the plasmid either from a plasmid-carrying cell from the same strain as the sampled infected individual, with probability $\left(\frac{1}{N}\right)$ or from a plasmid-carrying cell from a strain different to both of the ancestral strains of the sampled individuals with probability $\left(\frac{N-2}{N}p\right)$. The sampled plasmid-carrying individual is not affected by transmission. From these probabilities, the overall conditional probability for transmission is:

$$t_{pp\leftarrow p0} = \beta\left(\frac{1}{N} + \frac{N-2}{N}p\right).$$

As only one cell is infected with the plasmid (as the other cell already carries the plasmid), β only appears once in the above equation.

Pair probabilities

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables A2 and A3) and substituting into equation (A3), which gives:

$$\begin{aligned}\mathrm{E}[p_j^{\,t} p_j^{\,t}] = {} & \frac{1}{N}p \\ & + \frac{N-1}{N}p^2 \\ & + \frac{1}{N}(1-p)\beta^2\left(\frac{1}{N}\frac{N-1}{N}p + \frac{N-1}{N}\frac{N-2}{N}p^2\right) \\ & + \frac{N-1}{N}(1-p)^2\beta^2\left(\frac{1}{N}\frac{N-2}{N}p + \frac{N-2}{N}\frac{N-3}{N}p^2\right) \\ & + 2\frac{N-1}{N}p(1-p)\beta\left(\frac{1}{N} + \frac{N-2}{N}p\right).\end{aligned} \qquad \text{[A5]}$$

Supplementary tables for Chapter 4 Appendix A

Table A1. Moments required for calculation of $E[p_{ij}^{\,t} p_{ij}^{\,t} p_j^{\,t}]$

All sampling performed among founders before transmission.

Moment	Definition	Probability
$E[p_{ij}p_j]$	Probability of sampling two plasmid carriers	$Np(1+(N\text{-}1)p)$
$E[p_{ij}p_jp_j]$ or $E[p_jp_jp_j]$	Probability of sampling three plasmid carriers	$Np(1+(N\text{-}1)p(3+(N\text{-}2)p))$
$E[p_{ij}p_jp_jp_j]$or $E[p_jp_jp_jp_j]$	Probability of sampling four plasmid carriers	$Np(1+(N\text{-}1)p(7+(N\text{-}2)p(6+(N\text{-}3)p)))$
$E[p_{ij}p_jp_jp_jp_j]$	Probability of sampling five plasmid carriers	$Np(1+(N\text{-}1)p\ (15+\ (N\text{-}2)p(25+(N\text{-}3)p(10+\ (N\text{-}4)p))))$

Table A2. State of origin probabilities, x_i for equation (A4).

x_i	Probability
x_p	$\frac{1}{N}p$
x_{pp}	$\frac{N-1}{N}p^2$
x_0	$\frac{1}{N}(1-p)$
x_{00}	$\frac{N-1}{N}(1-p)^2$
x_{p0}	$2\frac{N-1}{N}p(1-p)$

Table A3. The calculation of $t_{pp\leftarrow i}$ for equation (A4).

$t_{pp\leftarrow i}$	Transition probability
$t_{pp\leftarrow p}$	1
$t_{pp\leftarrow pp}$	1
$t_{pp\leftarrow 0}$	$\beta^2\left(\frac{1}{N}\frac{N-1}{N}p+\frac{N-1}{N}\frac{N-2}{N}p^2\right)$
$t_{pp\leftarrow 00}$	$\beta^2\left(\frac{1}{N}\frac{N-2}{N}p+\frac{N-2}{N}\frac{N-3}{N}p^2\right)$
$t_{pp\leftarrow p0}$	$\beta\left(\frac{1}{N}+\frac{N-2}{N}p\right)$

Chapter 4 Appendix B: Supplementary Information for the Effects of Segregation

Full model derivation

The model is derived as before using equations (2) and (4-5) and substituting $\mathrm{E}[\Delta p_{ij}] = -sp.$ as described in the main text to give:

$$\Delta p = \frac{1}{w}\left(-\mathrm{Var}[p^t]\left(C_1 + vR^t\right) - v\mathrm{E}[p_{ij}{}^t p_j{}^t]p^t + v\mathrm{E}[p_{ij}{}^t p_{ij}{}^t p_j{}^t]\right) - sp. \qquad \text{[B1]}$$

Calculation of variance and $\mathrm{E}[p_{ij}{}^t p_{ij}{}^t p_j{}^t]$ in the absence of horizontal transmission and the presence of segregation

We begin with $\mathrm{E}[p_{ij}{}^t]$ which is calculated as $\mathrm{E}[p_{ij}{}^t] = p_{ij}(1-s)$. We then have

$\mathrm{E}[p_{ij}{}^t p_{ij}{}^t] = \left(p_{ij} - sp_{ij}\right)^2 = \mathrm{E}(p_{ij} - 2sp_{ij} + s^2 p_{ij}) = p - 2sp + s^2 p$. Variance is then calculated as in equation (A2). Relatedness R^t is calculated as in equation (A1) in appendix A.

Where is calculated $\mathrm{E}[p_{ij}{}^t p_{ij}{}^t p_j{}^t]$ as: $\mathrm{E}[p_{ij}{}^t p_{ij}{}^t p_j{}^t] = \left(p_{ij} - sp_{ij}\right)\left(p_{ij} - sp_{ij}\right)\left(p_j - sp_j\right)$. This gives $\mathrm{E}[p_{ij}{}^t p_{ij}{}^t p_j{}^t] = \mathrm{E}[p_{ij}p_j] - 3s\,\mathrm{E}[p_{ij}p_j] + 3s^2\,\mathrm{E}[p_{ij}p_j] - s^3\,\mathrm{E}[p_{ij}p_j]$. The moment, $\mathrm{E}[p_{ij}p_j]$, required for calculation of $\mathrm{E}[p_{ij}{}^t p_{ij}{}^t p_j{}^t]$ is shown in Table A1.

Within patch associations $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$ in the absence of horizontal transmission and the presence of segregation

We calculate $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$ using the same method illustrated in appendix A. As before, we can expand $\mathrm{E}[p_j^{\,t} p_j^{\,t}]$ in terms of conditional probabilities to give:

$$\mathrm{E}[p_j^{\,t} p_j^{\,t}] = \sum_{i \in s} \left(t_{pp \leftarrow i} x_i \right), \qquad \text{[B2]}$$

where $S \in \{p, pp, 0, 00, p0\}$. The state of origin probabilities (x_i) are described in Appendix A and listed in table A2 in Appendix A. The transition probabilities, $t_{pp \leftarrow i}$, are calculated below and listed in table B1.

Transition probabilities, $t_{pp \leftarrow i}$

1. Transition probability $t_{pp \leftarrow p}$

The first transition probability, $t_{pp \leftarrow p}$, gives the probability that two randomly sampled individuals which descend from the same plasmid-carrying strain, carry the plasmid after transmission. As transmission is absent in this model both individuals will remain plasmid carriers after the segregation stage provided they do not segregate their plasmids. This occurs with probability $(1-s)^2$. Thus $t_{pp \leftarrow p} = (1-s)^2$.

2. *Transition probability* $t_{pp\leftarrow pp}$

The second transition probability, $t_{pp\leftarrow pp}$, describes the probability that two randomly sampled individuals descending from separate plasmid-carrying strains, carry the plasmid after transmission. As before transmission is absent and both individuals will remain plasmid carriers after the segregation stage with probability $(1-s)^2$. Thus $t_{pp\leftarrow pp} = (1-s)^2$.

3. Transition probability $t_{pp\leftarrow 0}$

The transition probability, $t_{pp\leftarrow 0}$, gives the probability that two randomly sampled individuals, which descend from the same plasmid-free strain, carry the plasmid after transmission. As there is no transmission in this model the plasmid-free cells cannot be infected with the plasmid thus this probability is zero giving $t_{pp\leftarrow 0} = 0.$

4. *Transition probability* $t_{pp\leftarrow 00}$

The transition probability, $t_{pp\leftarrow 00}$, gives the probability that two randomly sampled individuals, which descend from different plasmid-free strains, carry the plasmid after transmission. As there this probability is zero as there is no transmission in this model giving $t_{pp\leftarrow 00} = 0.$

5. *Transition probability* $t_{pp\leftarrow p0}$

The transition probability, $t_{pp\leftarrow p0}$, describes the probability that two randomly sampled individuals, one descending from a plasmid-free strain and the other from a plasmid-carrying strain, carry the plasmid after transmission. The transition $t_{11\leftarrow 10}$ occurs with probability zero as transmission is absent in this model therefore the plasmid-free cell cannot gain a plasmid giving $t_{pp\leftarrow p0} = 0.$

Pair probabilities

We calculate the overall probabilities by combining the conditional probabilities given above (and in tables A2 and B1) substituting them into equation (B2) to give:

$$\mathrm{E}[p_j{}^{\mathrm{t}} p_j{}^{\mathrm{t}}] = \frac{1}{N} p(1-s)^2 + \frac{N-1}{N} p^2(1-s)^2. \qquad \text{[B3]}$$

We then substitute equation (B3) into our expression for relatedness (A1) and substitute p^t for $p^t = (1-s)p$ (as described in the main text) to obtain the value for R^t.

Supplementary Tables for Chapter 4 Appendix B

Table B1. The calculation of transition probabilities, $t_{pp\leftarrow i}$ in the absence of horizontal gene transfer and the presence of segregation.

$t_{pp\leftarrow i}$	Probability
$t_{pp\leftarrow p}$	$(1-s)^2$
$t_{pp\leftarrow pp}$	$(1-s)^2$
$t_{pp\leftarrow 0}$	0
$t_{pp\leftarrow 00}$	0
$t_{pp\leftarrow p0}$	0

Chapter 5 Appendix A: Supplementary materials for resistance model

Model Structure

We use a standard population genetical approach, and derive our model from the Price Equation (Price 1970; Price 1972) in order to evaluate the change in frequency *p* of the plasmid in the population. This is given as equation (1) in the main text:

$$\underbrace{\Delta p_x}_{\text{Change in gene frequency}} = \underbrace{\frac{1}{w}\text{Cov}[w_{ij}, p_{xij}]}_{\text{Selection}} + \underbrace{\frac{1}{w}\text{E}[w_{ij}, \Delta p_{xij}]}_{\text{Transmission}} \qquad \text{[A1]}$$

As in previous work (Grafen 1985; Taylor 1990; Taylor 1996; Grafen 2008), the expectation $\text{E}[\cdot]$ denotes an average of a quantity over all individuals in the population; that is the sum over all individuals within a patch (*i*) summed over all patches (*j*) and divided by the total number of individuals in the population. For instance, the average plasmid frequency in the population is $p = \text{E}[p_{ij}] = \sum_{ij} p_{ij}/(Nn)$ where *n* is the number of patches in the population (assumed infinite) and the average fitness is $w = \text{E}[w_{ij}] = \sum_{ij} w_{ij}/(Nn)$. We use the covariance $\text{Cov}[\cdot,\cdot]$ to denote the average over all individuals of the product of two quantities minus the product of the averages; for instance for the covariance in equation (A1), we have $\text{Cov}[w_{ij}, p_{ij}] = \text{E}[w_{ij} p_{ij}] - \text{E}[p_{ij}]\text{E}[w_{ij}]$.

Because of our life cycle assumptions, fitness, w_{ij}, refers to individual fitness after transmission, i.e. $w_{ij} = w_{ij}\left(p_{ij}^{\,t}, p_j^{\,t}\right)$ where $p_{ij}^{\,t}$ is an indicator variable of whether a randomly sampled, cell descending from a founder individual *i* in patch *j* carries the plasmid after transmission (this is a random variable) and $p_j^{\,t} = \sum_i p_{ij}^{\,t} / N$ is the average frequency of the plasmid in patch *j*. This is also a random variable. Thus we have:

$$w\Delta p = \mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] - \mathrm{Cov}[w_{ij}, \Delta p_{ij}] + \mathrm{E}[w_{ij}\Delta p_{ij}].$$

We can then expand $\mathrm{Cov}[w_{ij}, \Delta p_{ij}]$ in terms of expectations to give:

$$w\Delta p = \mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] - (\mathrm{E}[w_{ij}, \Delta p_{ij}] - \mathrm{E}[w_{ij}]\mathrm{E}[\Delta p_{ij}]) + \mathrm{E}[w_{ij}\Delta p_{ij}].$$

As $w = \mathrm{E}[w_{ij}]$ we can now express equation (1) in terms of $p_{ij}^{\,t}$ as:

$$\Delta p = \frac{1}{w}\mathrm{Cov}[w_{ij}, p_{ij}^{\,t}] + \mathrm{E}[\Delta p_{ij}] \qquad [A2]$$

Equation (2) is the basis of our model calculations in the main text.

Resistance to plasmids – selection

Substituting w_{ij} and w into equation (2) in the main text, and rearranging gives the change, over one generation, in plasmid and resistance frequency from one generation to the next, in terms of p_x and $p_x^{\,t}$:

$$\Delta p_1 = \frac{1}{w}\left(B(\mathrm{E}[p_{1ij}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}] - p_1{}^{t2}) - C_1 p_1{}^{\mathrm{t}}(1 - p_1{}^{\mathrm{t}}) + C_2 p_1{}^{\mathrm{t}} p_2{}^{\mathrm{t}}\right) + \mathrm{E}[\Delta p_{1ij}] \qquad \text{[A3a]}$$

$$\Delta p_2 = \frac{1}{w}\left(B(\mathrm{E}[p_{2ij}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}] - p_1{}^{t} p_2{}^{\mathrm{t}}) + C_1 p_1{}^{\mathrm{t}} p_2{}^{\mathrm{t}} - C_2 p_2{}^{\mathrm{t}}(1 - p_2{}^{\mathrm{t}})\right) + \mathrm{E}[\Delta p_{2ij}]. \qquad \text{[A3b]}$$

In equation (A3a) we have

$$\mathrm{E}[p_{1ij}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}] = \lim_{n\to\infty}\left[\sum_{ij}\frac{p_{1ij}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}}{nN}\right] = \lim_{n\to\infty}\left[\sum_{j}\frac{p_{1j}{}^{\mathrm{t}}}{n}\sum_{i}\frac{p_{1ij}{}^{\mathrm{t}}}{N}\right] = \lim_{n\to\infty}\left[\sum_{j}\frac{p_{1j}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}}{n}\right] = \mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}]$$

$\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}]$ gives probability that two randomly sampled individuals from a randomly sampled patch will carry the plasmid. We also have, in equation (A3b):

$$\mathrm{E}[p_{2ij}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}] = \lim_{n\to\infty}\left[\sum_{ij}\frac{p_{1j} p_{2ij}}{nN}\right] = \lim_{n\to\infty}\left[\sum_{j} p_{1j}\sum_{i}\frac{p_{2ij}}{nN}\right] = \lim_{n\to\infty}\left[\sum_{ij}\frac{p_{2j} p_{1j}}{n}\right] = \mathrm{E}[p_{1j} p_{2j}].$$

$\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{2j}{}^{\mathrm{t}}]$ gives the probability that among two randomly sampled individuals, the first individual (tghe second individual) carries the plasmid (p_1) while the second individual (the first individual) carries the resistance allele (p_2), but since each individual could carry either, the probability that, among two randomly sampled individuals, one carries the plasmid and the other the resistance allele is $2\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{2j}{}^{\mathrm{t}}]$.

The within-patch association terms, $\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}]$ and $\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{2j}{}^{\mathrm{t}}]$, are calculated below.

Relatedness (R) and assortment (Q_{12})

We can express $\mathrm{E}[p_{1j}{}^{t} p_{1j}{}^{t}]$ in terms of whole group relatedness (R) as

$\mathrm{E}[p_{1j}{}^{t} p_{1j}{}^{t}] = Rp_1{}^{t} + (1-R)p_1{}^{t^2}$. We define R as the relatedness between a focal individual and another random individual from the same patch (sampled with replacement). Relatedness is the regression of the plasmid frequency in a host on the frequency of the plasmid in a focal founder:

$$R = \frac{\mathrm{E}[p_{1j}{}^{t} p_{1j}{}^{t}] - p_1{}^{t^2}}{p_1{}^{t}(1 - p_1{}^{t})} \qquad [A4]$$

Using equation (A4) we can express equation (S3a) as equation (3a) in the main text.

We express the association between plasmid carriers and resistance carriers as Q_{12} where

$$Q_{12} = \frac{\mathrm{E}[p_{1j}{}^{t} p_{2j}{}^{t}]}{p_1{}^{t} p_2{}^{t}} - \frac{p_1{}^{t} p_2{}^{t}}{p_1{}^{t} p_2{}^{t}}, \qquad [A5]$$

so that

$$Q_{12} = \frac{\mathrm{Cov}[p_{1j}{}^{t} p_{2j}{}^{t}]}{p_1{}^{t} p_2{}^{t}}..$$

Employing Q_{12} allows us to express equation (A3b) as equation (3b) in the main text.

Within-patch associations ($\mathrm{E}[p_{1j}{}^{t} p_{1j}{}^{t}]$ **and** $\mathrm{E}[p_{1j}{}^{t} p_{2j}{}^{t}]$ **).**

We measure the within patch associations between plasmid carriers and other plasmids carriers and between plasmid carriers and resistance carriers as $\mathrm{E}[p_{1j}{}^{t} p_{1j}{}^{t}]$ and $\mathrm{E}[p_{1j}{}^{t} p_{2j}{}^{t}]$ respectively.

Calculation of $\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{1j}{}^{\mathrm{t}}]$

The expectation $\mathrm{E}[p_j{}^{\mathrm{t}} p_j{}^{\mathrm{t}}]$ is the average over all patches of the square of the average plasmid frequency in each patch. It gives the probability that two randomly sampled individuals from a randomly sampled patch will carry the plasmid. We now expand this probability in terms of conditional probabilities as:

$$\mathrm{E}[p_j{}^{\mathrm{t}} p_j{}^{\mathrm{t}}] = \sum_{i \in s} \left(t_{11 \leftarrow i} x_i \right) , \tag{A6}$$

where $t_{11 \leftarrow i}$ is the probability that two plasmid-carrying individuals, randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of origin *i* before transmission (state of the cell before transmission), x_i is the probability of occurrence of the state of origin *i* and $S = \{1,11,2,22,0,00,10,20,12\}$. The index *i* represents the original state of the two cells, randomly sampled from the population, before transmission: *i*=1 refers to the case where both have the plasmid (and share the same founder cell), *i*=11 refers to the case where both have the plasmid (but come from different founder cells), *i*=2 refers to the case where both have the resistance gene (and share the same founder cell), *i*=22 refers to the case where both have the resistance gene (but come from different founder cells), *i*=0 refers to the case where both are plasmid- and resistance-free (and share the same founder cell), *i*=00 refers to the case where both are plasmid- and resistance-free (but come from different founder cells), *i*=10 refers to the case where one has the plasmid and the other is plasmid- and resistance-free (and hence they both come from different founder cells), *i*=20 refers to the case where one has the resistance gene and the other is plasmid-and resistance-free (both coming from different founder cells) and *i*=12 refers to the case where one has the plasmid and the other has the resistance gene (both coming from different founder cells). We now evaluate explicitly the probabilities going into equation (A6).

State of origin probabilities

The probability of occurrence of the state of origin *i* of the two descendant individuals is given by $x_{i,}$ where $i \in S$.

The probability that the two individuals sampled both descend from the same plasmid-carrying founder strain is given by $x_1 = \frac{1}{N} p_1$.

The probability that the two individuals sampled descend from two separate plasmid-carrying founder strains is $x_{11} = \frac{N-1}{N} {p_1}^2$.

The probability that the two individuals sampled both descend from the same resistance-carrying founder strain is given by $x_2 = \frac{1}{N} p_2$.

The probability that the two individuals sampled descend from two separate resistance -carrying founder strains is $x_{22} = \frac{N-1}{N} {p_2}^2$.

The probability that the two individuals sampled both descend from the same plasmid- and resistance-free founder strain is given by $x_0 = \frac{1}{N}(1 - p_1 - p_2)$.

The probability that the two individuals sampled descend from two separate plasmid- and resistance-free founder strains is given by $x_{00} = \frac{N-1}{N}(1 - p_1 - p_2)^2$.

The probability that, of the two individuals sampled, one descends from a plasmid-carrying strain and the other from a plasmid- and resistance-free strain is given by

$$x_{1\ 0} = 2\frac{N-1}{N} p_1(1 - p_1 - p_2).$$

The probability that, of the two individuals sampled, one descends from a resistance-carrying strain and the other from a plasmid- and resistance-free strain is given by

$$x_{20} = 2\frac{N-1}{N} p_2(1 - p_1 - p_2).$$

Finally, the probability that, of the two individuals sampled, one descends from a plasmid-carrying strain and the other from a resistance-carrying strain is given by: $x_{12} = 2\frac{N-1}{N} p_1 p_2$.

All of these probabilities are summarized in table A1. Below we consider each within-patch pair identity in turn.

Transition probabilities

The transition probabilities, which are listed in table A2, are as follows:

1. *Transition probability,* $t_{11\leftarrow 1}$

The first transition probability, $t_{11\leftarrow 1}$, gives the probability that two randomly sampled individuals which descend from the same plasmid-carrying strain, carry the plasmid after transmission. As both individuals already carry the plasmid this occurs with probability 1, giving $t_{11\leftarrow 1} = 1$.

2. Transition probability $t_{11\leftarrow 11}$

The second transition probability, $t_{11\leftarrow 11}$, describes the probability that two randomly sampled individuals descending from separate plasmid-carrying strains, carry the plasmid after transmission. This also occurs with probability 1, giving $t_{11\leftarrow 11} = 1$.

3. *Transition probability,* $t_{11\leftarrow 2}$

The first transition probability, $t_{11\leftarrow 2}$, gives the probability that two randomly sampled individuals which descend from the same resistance-carrying strain, carry the plasmid after transmission. As individuals carrying the resistance gene cannot be infected by the plasmid, this gives $t_{11\leftarrow 2} = 0$.

4. Transition probability $t_{11\leftarrow 22}$

The second transition probability, $t_{11\leftarrow 22}$, describes the probability that two randomly sampled individuals descending from separate resistance-carrying strains, carry the plasmid after transmission. As before, the plasmid cannot infect a resistant cell, and thus $t_{11\leftarrow 22} = 0$.

5. Transition probability $t_{11\leftarrow 0}$

The transition probability, $t_{11\leftarrow 0}$, gives the probability that two randomly sampled individuals, which descend from the same plasmid- and resistance-free strain, carry the plasmid after transmission. Both of the individuals sampled, as they do not carry the resistance gene, may be infected upon contact with a plasmid-carrying individual at the transmission stage with probability β^2. All such plasmid-carrying strains are different to the ancestral strain of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which descend from the same plasmid-carrying strain. This

occurs with probability $\left(\frac{1}{N}\frac{N-1}{N}p_1\right)$. Alternatively the sampled individuals may infected by plasmid-carrying cells which descend from two separate plasmid-carrying strains, this occurs with probability $\left(\frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right)$. Thus, the overall transition probability is given by:

$$t_{11\leftarrow 0} = \beta^2\left(\frac{1}{N}\frac{N-1}{N}p_1 + \frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right)$$

The β^2 is due to the fact that both cells must have been infected with a plasmid (which occurs with a probability β.

6. Transition probability $t_{11\leftarrow 00}$

The transition probability, $t_{11\leftarrow 00}$, describes the probability that two randomly sampled individuals descending from separate plasmid-and resistance-free strains, carry the plasmid after transmission. Both may be infected at the transmission stage upon contact with plasmid-carrying individuals at rate β^2. As above, all such plasmid-carrying strains are different from the ancestral strains of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which come from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-2}{N}p_1\right)$. Alternatively the sampled individuals may be infected by plasmid-carrying cells from two separate plasmid-carrying strains, this occurs with probability $\left(\frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right)$. From these the overall conditional probability is given by:

$$t_{11\leftarrow 00} = \beta^2\left(\frac{1}{N}\frac{N-2}{N}p_1 + \frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right).$$

7. Transition probability $t_{11\leftarrow 10}$

The transition probability, $t_{11\leftarrow 10}$, describes the probability that two randomly sampled individuals, one descending from a plasmid- and resistance-free strain and the other from a plasmid-carrying strain, carry the plasmid after transmission. Upon contact with a plasmid carrier the uninfected sampled individual acquires the plasmid with probability β. It may acquire a copy of the plasmid either from a plasmid-carrying cell from the same strain as the sampled infected individual, with probability $\left(\frac{1}{N}\right)$ or from a plasmid-carrying cell from a strain different to both of the ancestral strains of the sampled individuals with probability $\left(\frac{N-2}{N}p_1\right)$. The sampled plasmid-carrying individual is not affected by transmission. From these probabilities, the overall conditional probability for transmission is:

$$t_{11\leftarrow 10} = \beta\left(\frac{1}{N} + \frac{N-2}{N}p_1\right)$$

As only one cell is infected with the plasmid (as the other cell already carries the plasmid), β only appears once in the above equation.

8. Transition probability $t_{11\leftarrow 20}$

The second transition probability, $t_{11\leftarrow 20}$, describes the probability that two randomly sampled individuals, one descending from a plasmid- and resistance-free strain and the other from a resistance-carrying strain, carry the plasmid after transmission. As before, plasmids cannot infect resistant cells, and thus $t_{11\leftarrow 20} = 0$.

9. Transition probability $t_{11\leftarrow 12}$

The second transition probability, $t_{11\leftarrow 12}$, describes the probability that two randomly sampled individuals, one descending from a plasmid-carrying strain and the other from a resistance-carrying strain, carry the plasmid after transmission. As before, there is no plasmid spread into resistant cells, which gives $t_{11\leftarrow 12} = 0$.

Pair probabilities for $\mathrm{E}[{p_{1_j}}^{\mathrm{t}} {p_{1_j}}^{\mathrm{t}}]$

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables A1 and A2) using the following equation (A6). This then gives the following equation for the pair probabilities:

$$\begin{aligned}\mathrm{E}[{p_{1_j}}^{\mathrm{t}} {p_{1_j}}^{\mathrm{t}}] = {} & \frac{1}{N} p_1 \\ & + \frac{N-1}{N} {p_1}^2 \\ & + \frac{1}{N}(1-p_1-p_2)\beta^2\left(\frac{1}{N}\frac{N-1}{N} p_1 + \frac{N-1}{N}\frac{N-2}{N} {p_1}^2\right) \\ & + \frac{N-1}{N}(1-p_1-p_2)^2\beta^2\left(\frac{1}{N}\frac{N-2}{N} p_1 + \frac{N-2}{N}\frac{N-3}{N} {p_1}^2\right) \\ & + 2\frac{N-1}{N} p_1(1-p_1-p_2)\beta\left(\frac{1}{N} + \frac{N-2}{N} p_1\right).\end{aligned} \qquad [A7]$$

Calculation of $\mathrm{E}[{p_{2_{ij}}}^{\mathrm{t}} {p_{1_j}}^{\mathrm{t}}]$

In order to evaluate $\mathrm{E}[{p_{1_j}}^{\mathrm{t}} {p_{2_j}}^{\mathrm{t}}]$ we use P_{12}, which the probability that among two randomly sampled individuals, one carries plasmid 1 and the other carries plasmid 2 such that:

$$P_{12} = 2\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{2j}{}^{\mathrm{t}}].$$

We can expand P_{12} as $\sum_{i \in s} h_{12 \leftarrow i}.x_i$ which allows us to expand $\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{2j}{}^{\mathrm{t}}]$ as:

$$\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{2j}{}^{\mathrm{t}}] = \frac{1}{2} P_{12} = \frac{1}{2} \sum_{i \in s} g_{12 \leftarrow i}.x_i. \qquad [A8]$$

Equation (A8) consists of two probabilities, x_i and $g_{12 \leftarrow i}$. The probability of occurrence of the state of origin *i* of the two descendant individuals is given by $x_{i,}$ where $i \in S$ as described above and in table A1. The probability that one plasmid-carrying individual and resistant individual, sampled randomly after transmission, descend from state of origin *i* before transmission is given by $g_{12 \leftarrow i}$. This is the sum of the probability of randomly sampling first a plasmid-carrying individual followed by a resistance-carrying individual and the probability of randomly sampling first a resistance carrier and then a plasmid carrier. These two probabilities are equal (as described above).

Transition probabilities

The transition probabilities, which are listed in table A3, are as follows:

1. Transition probability $g_{12 \leftarrow 1}$

The transition $g_{12 \leftarrow 1}$ describes the probability that of two randomly sampled individuals, which descend from the same plasmid-carrying strain, one carries the plasmid and the other

carries resistance after transmission. As resistance cannot be horizontally transferred, thus $g_{12\leftarrow 1} = 0$.

2. Transition probability $g_{12\leftarrow 11}$

The transition $g_{12\leftarrow 11}$ describes the probability that of two randomly sampled individuals, which descend from different plasmid-carrying strains, one carries the plasmid and the other carries the resistance gene after transmission. As above, this probability is zero: $g_{12\leftarrow 11} = 0$.

3. Transition probability $g_{12\leftarrow 2}$

The transition $g_{12\leftarrow 2}$ describes the probability that of two randomly sampled individuals, which descend from the same resistance-carrying strain, one carries the plasmid and the other carries the resistance gene after transmission. As resistant individuals cannot be infected with the plasmid, this probability is zero: $g_{12\leftarrow 2} = 0$.

4. Transition probability $g_{12\leftarrow 22}$

The transition $g_{12\leftarrow 22}$ describes the probability that of two randomly sampled individuals, which descend from different resistant strains, one carries the plasmid and the other carries resistance after transmission. As above, this probability is zero: $g_{12\leftarrow 22} = 0$.

5. *Transition probability* $g_{12\leftarrow 0}$

Transition probability, $g_{12\leftarrow 0}$, describes the probability that of two randomly sampled individuals, which descend from the same plasmid- and resistance-free strain, one carries the plasmid and the other carries resistance after transmission. As resistance cannot be transmitted horizontally this gives $g_{12\leftarrow 0} = 0$.

6. Transition probability $g_{12\leftarrow 00}$

The transition $g_{12\leftarrow 00}$ describes the probability that of two randomly sampled individuals, which descend from different plasmid- and resistance-free strains, one carries the plasmid and the other carries resistance after transmission. As before, resistance cannot spread horizontally, giving $g_{12\leftarrow 00} = 0.$

7. Transition probability $g_{12\leftarrow 10}$

The transition $g_{12\leftarrow 10}$ describes the probability that of two randomly sampled individuals, which descend from different strains (one a plasmid-carrier, the other plasmid- and resistance-free), one carries the plasmid and the other carries resistance after transmission. As above, resistance cannot be transmitted horizontally, giving $g_{12\leftarrow 10} = 0.$

8. *Transition probability* $g_{12\leftarrow 20}$

Transition $g_{12\leftarrow 20}$ describes the probability that of two randomly sampled individuals, which descend from different strains (one a resistance-carrier, the other plasmid- and resistance-free), one carries the plasmid and the other carries resistance after transmission. The plasmid-free individual may gain plasmid 1 at the transmission stage. Contact with a plasmid-carrying individual occurs with probability $\left(\frac{N-2}{N} p_1\right)$ and transfer of the plasmid occurs with probability β. This gives $g_{12\leftarrow 20} = \beta\left(\frac{N-2}{N} p_1\right).$

9. *Transition probability* $g_{12\leftarrow 12}$

Transition probability $g_{12\leftarrow 12}$ describes the probability that of two randomly sampled individuals, which descend from different strains (one a plasmid-carrier, the other a

resistance-carrier), one carries the plasmid and the other carries the resistance gene after transmission. As this is already the case before transmission, this probability is one: $g_{12\leftarrow 12} = 1$.

Pair probabilities for $\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{2j}{}^{\mathrm{t}}]$

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables A1 and A3) and using equation (A8), which gives

$$\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{2j}{}^{\mathrm{t}}] = \frac{1}{2}\left(\begin{array}{l} 2\frac{N-1}{N} p_2(1-p_1-p_2)\beta\left(\frac{N-2}{N} p_1\right) \\ +2\frac{N-1}{N} p_1 p_2 \end{array}\right). \qquad [A9]$$

Chapter 5 Appendix A Supplementary Tables

Table A1. The calculation of x_i for model 1.

x_i	Probability
x_1	$\frac{1}{N} p_1$
x_{11}	$\frac{N-1}{N} {p_1}^2$
x_2	$\frac{1}{N} p_2$
x_{22}	$\frac{N-1}{N} {p_2}^2$
x_0	$\frac{1}{N}(1-p_1-p_2)$
x_{00}	$\frac{N-1}{N}(1-p_1-p_2)^2$
x_{10}	$2\frac{N-1}{N} p_1(1-p_1-p_2)$
x_{20}	$2\frac{N-1}{N} p_2(1-p_1-p_2)$
x_{12}	$2\frac{N-1}{N} p_1 p_2$

Table A2. The calculation of $t_{11\leftarrow i}$ for model 1.

$t_{11\leftarrow i}$	Transition probability
$t_{11\leftarrow 1}$	1
$t_{11\leftarrow 11}$	1
$t_{11\leftarrow 2}$	0
$t_{11\leftarrow 22}$	0
$t_{11\leftarrow 0}$	$\beta^2\left(\frac{1}{N}\frac{N-1}{N}p_1+\frac{N-1}{N}\frac{N-2}{N}p_1^{\,2}\right)$
$t_{11\leftarrow 00}$	$\beta^2\left(\frac{1}{N}\frac{N-2}{N}p_1+\frac{N-2}{N}\frac{N-3}{N}p_1^{\,2}\right)$
$t_{11\leftarrow 10}$	$\beta\left(\frac{1}{N}+\frac{N-2}{N}p_1\right)$
$t_{11\leftarrow 20}$	0
$t_{11\leftarrow 12}$	0

Table A3. The calculation of $g_{12\leftarrow i}$ for model 1.

$g_{12\leftarrow i}$	Transition probability
$g_{12\leftarrow 1}$	0
$g_{12\leftarrow 11}$	0
$g_{12\leftarrow 2}$	0
$g_{12\leftarrow 22}$	0
$g_{12\leftarrow 0}$	0
$g_{12\leftarrow 00}$	0
$g_{12\leftarrow 10}$	0
$g_{12\leftarrow 20}$	$\beta\left(\frac{N-2}{N}p_1\right)$
$g_{12\leftarrow 12}$	1

Chapter 5 Appendix B: Supplementary materials for suppressor model

Suppression of plasmid gene expression - selection

An individual benefits from the public goods produced by itself (if it carries the plasmid) and by other plasmid carrying individuals in the population. Individuals carrying the suppressor exhibit reduced production of the public good (and reduced associated costs). This is described by the parameter *h*, which ranges from zero to one, where *h*=1 results in full expression of the public good and *h*=0 prevents expression of the cooperative gene entirely. Thus an individual *i* receives benefit:

$$w_{ij}^{\ t} = 1 + Bp_{1j}^{\ t}\left(1 - hp_{2j}^{\ t}\right) - Cp_{1ij}^{\ t}\left(1 - hCp_{2ij}\right) - C_2 p_{2ij}^{\ t} - vp_{1ij}^{\ t}.$$

$$B\frac{1}{N}\sum_{i=1}^{N} p_{1ij}^{\ t} - hB\frac{1}{N}\sum_{i=1}^{N} p_{1ij}^{\ t} p_{2ij}^{\ t} = Bp_{1j}^{\ t} - hBp_{12j}^{\ t}.$$

Therefore, employing the fitness function $w_{ij}^{\ t}$, we have:

$$\mathrm{Cov}(w_{ij}^{\ t} p_{1ij}^{\ t}) = \mathrm{Cov}\left((1 + Bp_{1j}^{\ t}\left(1 - hp_{2j}^{\ t}\right) - Cp_{1ij}^{\ t}\left(1 - hCp_{2ij}\right) - C_2 p_{2ij}^{\ t} - vp_{1ij}^{\ t})\, p_{1ij}^{\ t}\right),$$

which gives:

$$\mathrm{Cov}(w_{ij}^{\ t} p_{1ij}^{\ t}) = B\mathrm{Cov}(p_{1j}^{\ t}, p_{1ij}^{\ t}) - hB\mathrm{Cov}(p_{12j}^{\ t}, p_{1ij}^{\ t}) - C\mathrm{Cov}(p_{1ij}^{\ t}, p_{1ij}^{\ t})$$
$$+ hC\mathrm{Cov}(p_{1ij}^{\ t} p_{2ij}^{\ t}, p_{1ij}^{\ t}) - C_2\mathrm{Cov}(p_{2ij}^{\ t}, p_{1ij}^{\ t}) - x\mathrm{Cov}(p_{1ij}^{\ t}, p_{1ij}^{\ t}).$$

Because $\mathrm{Cov}[X,Y] = \mathrm{E}[XY] - \mathrm{E}[X]\mathrm{E}[Y]$, we can simplify the above into a series of calculations as follows:

$$B\mathrm{Cov}({p_{1j}}^t, {p_{1ij}}^t) = B\left(\mathrm{E}[{p_{1j}}^t {p_{1ij}}^t] - \mathrm{E}[{p_{1j}}^t]E[{p_{1ij}}^t]\right) = B\left(\mathrm{E}[{p_{1j}}^t {p_{1ij}}^t] - p_1^{t2}\right),$$

$$hB\mathrm{Cov}({p_{12j}}^t, {p_{1ij}}^t) = hB\left(\mathrm{E}[{p_{1j}}^t {p_{12ij}}^t] - \mathrm{E}[{p_{12j}}^t]\mathrm{E}[{p_{1ij}}^t]\right)$$
$$= hB\left(\mathrm{E}[{p_{12j}}^t {p_{1ij}}^t] - p_1^{t2} p_2^t\right)$$

$$C\mathrm{Cov}({p_{1ij}}^t, {p_{1ij}}^t) = Cp_1^t(1 - p_1^t),$$

$$hC\mathrm{Cov}({p_{1ij}}^t {p_{2ij}}^t, {p_{1ij}}^t) = hC\left(\mathrm{E}[{p_{1ij}}^t {p_{2ij}}^t {p_{1ij}}^t] - \mathrm{E}[{p_{1ij}}^t {p_{2ij}}^t]\mathrm{E}[{p_{1ij}}^t]\right)$$
$$= hCp_1^t p_2^t(1 - p_1^t),$$

$$C_2\mathrm{Cov}({p_{2ij}}^t, {p_{1ij}}^t) = 0,$$

and $x\mathrm{Cov}({p_{1ij}}^t, {p_{1ij}}^t) = xp_1^t(1 - p_1^t)$.

Combining these terms we get:

$$\mathrm{Cov}({w_{ij}}^t, {p_{1ij}}^t) = B\left(\mathrm{E}[{p_{1j}}^t {p_{1ij}}^t] - p_1^{t2}\right) - hB\left(\mathrm{E}[{p_{12j}}^t {p_{1ij}}^t] - p_1^{t2} p_2^t\right) - Cp_1^t(1 - p_1^t) +$$
$$hCp_1^t p_2^t(1 - p_1^t) - xp_1^t(1 - p_1^t).$$

We can calculate $\mathrm{Cov}({w_{ij}}^t, {p_{2ij}}^t)$ in a similar fashion.

Relatedness and assortment

Relatedness is calculated as $R = \frac{\mathrm{E}[{p_{1j}}^{t} {p_{1j}}^{t}] - {p_1}^{t^2}}{{p_1}^{t}(1 - {p_1}^{t})}$ (as described in appendix A). As we find $\mathrm{E}[{p_{1ij}}^{t} {p_{12j}}^{t}] = \mathrm{E}[{p_{1ij}}^{t} {p_{1j}}^{t}]{p_2}^{t}$ we can rephrase $B\left(\mathrm{E}[{p_{1ij}}^{t} {p_{12j}}^{t}] - {p_1}^{t^2} {p_2}^{t}\right)$ as $B\left(\mathrm{E}[{p_{1ij}}^{t} {p_{1j}}^{t}]{p_2}^{t} - {p_1}^{t^2} {p_2}^{t}\right)$ giving $B{p_2}^{t}\left({p_1}^{t}(1 - {p_1}^{t})R\right)$ in equation (6). We also calculate Q_{12} as seen in Appendix A as $Q_{12} = \frac{\mathrm{Cov}[{p_{1j}}^{t} {p_{2j}}^{t}]}{{p_1}^{t} {p_2}^{t}}$.

Carriers of the plasmid and the suppressor

For notational clarity we denote those individuals who carry both alleles with the subscript 3 such that the indicator variable p_{12ij}, which takes the value 1 if the individual carries both the plasmid and the suppressor and zero otherwise, is denoted by p_{3ij}. The average of this value across patches is denoted p_{3j} and the average frequency, across the population, of carriers of both traits is denoted p_3 which is calculated as $p_2 p_1$. We calculate the association between suppressor carriers and cells which carry both the suppressor and the plasmid in the same way as we calculated Q_{12} giving $Q_{23} = \frac{\mathrm{Cov}[{p_{2j}}^{t} {p_{3j}}^{t}]}{{p_2}^{t} {p_3}^{t}} = \frac{\mathrm{E}[{p_{2j}}^{t} {p_{3j}}^{t}] - {p_2}^{t} {p_3}^{t}}{{p_2}^{t} {p_3}^{t}}$.

Within-patch associations

($\mathrm{E}[{p_{1j}}^{t} {p_{1j}}^{t}]$**,** $\mathrm{E}[{p_{2j}}^{t} {p_{1j}}^{t}]$**,** $\mathrm{E}[{p_{1j}}^{t} {p_{3j}}^{t}]$ **and** $\mathrm{E}[{p_{2j}}^{t} {p_{3j}}^{t}]$ **)**

We measure the within patch associations between plasmid carriers and other plasmids carriers and between plasmid carriers and suppressor carriers as $\mathrm{E}[{p_{1j}}^{t} {p_{1j}}^{t}]$ and $\mathrm{E}[{p_{1j}}^{t} {p_{2j}}^{t}]$

respectively. In addition, we also measure the association between individuals which only carry the plasmid and individuals which carry both the plasmid and the suppressor ($\mathrm{E}[p_{1j}{}^{t} p_{3j}{}^{t}]$) as well as the association between individuals which only carry the suppressor and individuals which carry both the plasmid and the suppressor ($\mathrm{E}[p_{2j}{}^{t} p_{3j}{}^{t}]$).

Calculation of $\mathrm{E}[p_{1j}{}^{t} p_{1j}{}^{t}]$

The expectation $\mathrm{E}[p_{1j}{}^{t} p_{1j}{}^{t}]$ is the average over all patches of the square of the average plasmid frequency in each patch, and since the number of patches is infinite it gives the probability that two randomly sampled individuals from a randomly sampled patch will carry the plasmid. We now expand this probability in terms of conditional probabilities as:

$$\mathrm{E}[p_{1j}{}^{t} p_{1j}{}^{t}] = \sum_{i \in s} \left(t_{11 \leftarrow i} x_i \right) , \qquad \text{(B1)}$$

where $t_{11 \leftarrow i}$ is the probability that two plasmid-carrying individuals, randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of origin *i* before transmission (state of the cell before transmission), x_i is the probability of occurrence of the state of origin *i* and $S = \{1,11,0,00,10\}$. The index *i* represents the original state of the two cells, randomly sampled from the population, before transmission: *i*=1 refers to the case where both have the plasmid (and share the same founder cell), *i*=11 refers to the case where both have the plasmid (but come from different founder cells), *i*=0 refers to the case where both are plasmid-free, regardless of whether the cells carry the suppressor gene or

not (and share the same founder cell), *i*=00 refers to the case where both are plasmid-free, regardless of whether the cells carry the suppressor gene or not (but come from different founder cells) and finally, *i*=10 refers to the case where one has the plasmid and the other is plasmid –free, regardless of whether it carries the suppressor gene or not (and hence they both come from different founder cells. We now evaluate explicitly the probabilities going into equation (B1).

State of origin probabilities

The probability of occurrence of the state of origin *i* of the two descendant individuals is given by $x_{i,}$ where $i \in S$.

The probability that the two individuals sampled both descend from the same plasmid-carrying founder strain is given by $x_1 = \frac{1}{N} p_1$ .The probability that the two individuals sampled descend from two separate plasmid-carrying founder strains is $x_{11} = \frac{N-1}{N} p_1^{\,2}$.

The probability that the two individuals sampled both descend from the same plasmid-free founder strain is given by $x_0 = \frac{1}{N}(1 - p_1)$. The probability that the two individuals sampled descend from two separate plasmid-free founder strains is given by $x_{00} = \frac{N-1}{N}(1 - p_1)^2$.

The probability that, of the two individuals sampled, one descends from a plasmid-carrying strain and the other from a plasmid-free strain is given by $x_{10} = 2\frac{N-1}{N} p_1(1 - p_1)$. All of

these probabilities are summarized in table B1. Below we consider each within-patch pair identity in turn.

Transition probabilities

The transition probabilities, which are listed in table B2, are as follows:

1. *Transition probability,* $t_{11\leftarrow 1}$

The first transition probability, $t_{11\leftarrow 1}$, gives the probability that two randomly sampled individuals which descend from the same plasmid-carrying strain, carry the plasmid after transmission. As both individuals already carry the plasmid this occurs with probability 1, giving $t_{11\leftarrow 1} = 1$.

2. Transition probability $t_{11\leftarrow 11}$

The second transition probability, $t_{11\leftarrow 11}$, describes the probability that two randomly sampled individuals descending from separate plasmid-carrying strains, carry the plasmid after transmission. This also occurs with probability 1, giving $t_{11\leftarrow 11} = 1$.

3. Transition probability $t_{11\leftarrow 0}$

The transition probability, $t_{11\leftarrow 0}$, gives the probability that two randomly sampled individuals, which descend from the same plasmid-free strain, carry the plasmid after transmission. Both of the individuals sampled may be infected upon contact with a plasmid-carrying individual at the transmission stage with probability β^2. All such plasmid-carrying strains are different to the ancestral strain of the sampled individuals by virtue of their plasmid carriage. The

sampled individuals may both be infected by plasmid-carrying cells which descend from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-1}{N}p_1\right)$. Alternatively the sampled individuals may infected by plasmid-carrying cells which descend from two separate plasmid-carrying strains, this occurs with probability $\left(\frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right)$. Thus, the overall transition probability is given by:

$$t_{11\leftarrow 0} = \beta^2\left(\frac{1}{N}\frac{N-1}{N}p_1 + \frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right)$$

4. Transition probability $t_{11\leftarrow 00}$

The transition probability, $t_{11\leftarrow 00}$, describes the probability that two randomly sampled individuals descending from separate plasmid-free strains, carry the plasmid after transmission. Both may be infected at the transmission stage upon contact with plasmid-carrying individuals at rate β^2. As above, all such plasmid-carrying strains are different from the ancestral strains of the sampled individuals by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which come from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-2}{N}p_1\right)$. Alternatively the sampled individuals may be infected by plasmid 1-carrying cells from two separate plasmid-carrying strains, this occurs with probability $\left(\frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right)$. From these the overall conditional probability is given by:

$$t_{11\leftarrow 00} = \beta^2\left(\frac{1}{N}\frac{N-2}{N}p_1 + \frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right).$$

5. Transition probability $t_{11\leftarrow 10}$

The transition probability, $t_{11\leftarrow 10}$, describes the probability that two randomly sampled individuals, one descending from a plasmid-free strain and the other from a plasmid-carrying strain, carry the plasmid after transmission. Upon contact with a plasmid carrier the uninfected sampled individual acquires the plasmid with probability β. It may acquire a copy of the plasmid either from a plasmid-carrying cell from the same strain as the sampled infected individual, with probability $\left(\frac{1}{N}\right)$ or from a plasmid-carrying cell from a strain different to both of the ancestral strains of the sampled individuals with probability $\left(\frac{N-2}{N} p_1\right)$. The sampled plasmid-carrying individual is not affected by transmission. From these probabilities, the overall conditional probability for transmission is:

$$t_{11\leftarrow 10} = \beta\left(\frac{1}{N} + \frac{N-2}{N} p_1\right)$$

Pair probabilities for $\mathrm{E}[{p_{1_j}}^{t} {p_{1_j}}^{t}]$

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables B1 and B2) using equation (B1). This then gives the following equation for the pair probabilities:

$$
\begin{aligned}
\mathrm{E}[{p_j}^{\mathrm{t}}{p_{1j}}^{\mathrm{t}}] = {} & \frac{1}{N}p_1 \\
& + \frac{N-1}{N}{p_1}^2 \\
& + \frac{1}{N}(1-p_1)\beta^2\left(\frac{1}{N}\frac{N-1}{N}p_1 + \frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right) \\
& + \frac{N-1}{N}(1-p_1)^2\beta^2\left(\frac{1}{N}\frac{N-2}{N} + \frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right) \\
& + \frac{N-1}{N}2p_1(1-p_1)\beta\left(\frac{1}{N} + \frac{N-2}{N}p_1\right).
\end{aligned}
\qquad \text{[B2]}
$$

Calculation of $\mathrm{E}[{p_{1j}}^{\mathrm{t}}{p_{2j}}^{\mathrm{t}}]$

The expectation $\mathrm{E}[{p_{1j}}^{\mathrm{t}}{p_{2j}}^{\mathrm{t}}]$ gives the probability that, of two randomly sampled individuals from a randomly sampled patch, one will carry the plasmid and the other the suppressor gene. We now expand this probability in terms of conditional probabilities as:

$$
\mathrm{E}[{p_{1j}}^{\mathrm{t}}{p_{2j}}^{\mathrm{t}}] = \sum_{i\in s}\left(g_{12\leftarrow i}x_i\right), \qquad \text{(B3)}
$$

where $t_{11\leftarrow i}$ is the probability that two individuals (one plasmid-carrying and the other suppressor-carrying), randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of origin *i* before transmission (state of the cell before transmission), x_i is the probability of occurrence of the state of origin *i* and $S = \{2,22,3,33\}$. The index *i* represents the original state of the two cells, randomly sampled from the population, before transmission: *i*=2 refers to the case where both have the suppressor gene (but not the plasmid in this case) (and share the same founder cell), *i*=22

refers to the case where both have the suppressor gene (but not the plasmid) (but come from different founder cells), : *i*=3 refers to the case where both cells have both the suppressor gene and the plasmid (and share the same founder cell), and finally, *i*=33 refers to the case where both cells have both the suppressor gene and the plasmid (and come from different founder cells). As the suppressor gene cannot be transmitted horizontally, cells which do not contain the suppressor play no role in this calculation. We now evaluate explicitly the probabilities going into equation (B3).

State of origin probabilities

The probability of occurrence of the state of origin *i* of the two descendant individuals is given by $x_{i,}$ where $i \in S$. The probability that the two individuals sampled both descend from the same plasmid-free, suppressor-carrying founder strain is given by $x_2 = \frac{1}{N} p_2(1-p_1)$. The probability that the two individuals sampled descend from two separate plasmid-free, suppressor-carrying founder strains is $x_{22} = \frac{N-1}{N}\left(p_2(1-p_1)\right)^2$. The probability that the two individuals sampled both descend from the same plasmid- and suppressor-carrying founder strain is given by $x_3 = \frac{1}{N} p_1 p_2$. The probability that the two individuals sampled descend from two separate plasmid- and suppressor-carrying founder strains is given by $x_{33} = \frac{N-1}{N}(p_1 p_2)^2$. All of these probabilities are summarized in table B3. Below we consider each within-patch pair identity in turn.

Transition probabilities

The transition probabilities, which are listed in table B4, are as follows:

1. *Transition probability,* $g_{12\leftarrow 2}$

The first transition probability, $g_{12\leftarrow 2}$, gives the probability that, of two randomly sampled individuals which descend from the same plasmid-free, suppressor-carrying strain, one carries the suppressor and the other the plasmid after transmission. As one cell carries neither the suppressor nor the plasmid in order for this to occur this cell must be infected with the plasmid. This occurs with probability β upon contact with a plasmid carrier, which will come from a different strain to the sampled strain (as it carries a plasmid and the sampled strain does not) with probability $\left(\frac{N-1}{N} p_1\right)$. This gives an overall conditional probability for transmission of $g_{12\leftarrow 2} = \beta\left(\frac{N-1}{N} p_1\right)$.

2. Transition probability $g_{12\leftarrow 22}$

The second transition probability, $g_{12\leftarrow 22}$, describes the probability that, of two randomly sampled individuals which descend from separate plasmid-free, suppressor-carrying founder strains, one carries the suppressor and the other the plasmid after transmission. As above this depends on the infection of the plasmid-free, suppressor free cell with the plasmid. This will occur as before with probability β upon contact with a plasmid carrier. The plasmid carrier will necessarily come from a different strain to either of the sampled strains $\beta\left(\frac{N-2}{N} p_1\right)$, giving an overall probability of $g_{12\leftarrow 22} = \beta\left(\frac{N-2}{N} p_1\right)$.

3. Transition probability $g_{12\leftarrow 3}$

The transition probability, $g_{12\leftarrow 3}$, gives the probability that, of two randomly sampled individuals, which descend from the same plasmid-and suppressor-carrying strain, one will carry the plasmid and the other the suppressor after transmission. As both of the individuals sampled already carry both traits this gives $g_{12\leftarrow 3}=1.$

4. Transition probability $g_{12\leftarrow 33}$

The transition probability, $g_{12\leftarrow 33}$, describes the probability that, of two randomly sampled individuals, which descend from separate plasmid-and suppressor-carrying strains, one will carry the plasmid and the other the suppressor after transmission. As above, both of the individuals sampled already carry both traits thus giving $g_{12\leftarrow 33}=1.$

Pair probabilities for $\mathrm{E}[p_{1j}{}^{\mathrm{t}}\, p_{2j}{}^{\mathrm{t}}]$

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables B3 and B4) using equation (B3). This then gives the following equation for the pair probabilities:

$$\begin{aligned}\mathrm{E}[p_{1j}{}^{\mathrm{t}}\, p_{2j}{}^{\mathrm{t}}] = {} & \frac{1}{N}\, p_2(1-p_1)\beta\left(\frac{N-1}{N}\, p_1\right) \\ & + \frac{N-1}{N}\left(p_2(1-p_1)\right)^2 \beta\left(\frac{N-2}{N}\, p_1\right) \\ & + \frac{1}{N}(p_1 p_2) + \frac{N-1}{N}(p_1 p_2)^2.\end{aligned} \qquad [B4]$$

Calculation of $\mathrm{E}[p_{1j}{}^{t} p_{3j}{}^{t}]$

$\mathrm{E}[p_{1j}{}^{t} p_{3j}{}^{t}]$ is the probability that one samples a plasmid carrying individual (regardless of whether or not it also carriers the suppressor)and an individual who carries both the plasmid and the suppressor. As before expand this probability in terms of conditional probabilities as:

$$\mathrm{E}[p_{1j}{}^{t} p_{3j}{}^{t}] = \sum_{i \in s} \left(h_{13 \leftarrow i} x_i \right) , \tag{B5}$$

where $h_{13 \leftarrow i}$ is the probability that two individuals, one carrying the plasmid and the other carrying both the plasmid and the suppressor, randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of origin *i* before transmission (state of the cell before transmission), x_i is the probability of occurrence of the state of origin *i* and $S = \{3,13,03,12,02,2\}$. The index *i* represents the original state of the two cells, randomly sampled from the population, before transmission: *i*=3 refers to the case where both have the suppressor gene and the plasmid (and share the same founder cell), *i*=13 refers to the case where one has the plasmid and the other has both the plasmid and the suppressor gene (and the cells come from different founder cells), *i*=03 refers to the case where the cells descend from different founder cells, with one having both traits and the other being plasmid-free , *i*=12 refers to the case where the cells descend from different founder cells, where one carries the plasmid (regardless of whether it carries the suppressor also) and the other carries only the suppressor gene, *i*=02 refers to the case where the cells descend from different founder cells, where one cell is plasmid-free and the other carries only the suppressor gene and finally *i*=2 refers to the case where both carry the suppressor gene but not

the plasmid (and share the same founder cell). We now evaluate explicitly the probabilities going into equation (B5).

State of origin probabilities

The probability of occurrence of the state of origin *i* of the two descendant individuals is given by $x_{i,}$ where $i \in S$. The probability that the two individuals sampled both descend from the same plasmid- and suppressor-carrying founder strain is given by $x_3 = \frac{1}{N} p_1 p_2$ .The probability that the two individuals sampled descend from two separate founders strains where one has the plasmid and the other has both the plasmid and the suppressor gene is given by $x_{13} = \frac{N-1}{N}(p_1 p_2)(p_1)$ .The probability that the two individuals sampled descend from different founder cells, with one having both traits and the other being plasmid-free is given by $x_{03} = \frac{N-1}{N}(p_1 p_2)(1-p_1)$. The probability that the two individuals sampled descend from different founder cells, where one carries the plasmid (regardless of whether it carries the suppressor also) and the other carries only the suppressor gene is given by $x_{12} = \frac{N-1}{N}(p_1)\left(p_2(1-p_1)\right)$. The probability that the two individuals sampled descend from different founder cells, where one cell is plasmid-free and the other carries only the suppressor gene is given by $x_{02} = \frac{N-1}{N}(1-p_1)\left(p_2(1-p_1)\right)$. The probability that the two individuals sampled descend from the same founder cell and both carry the suppressor gene but not the plasmid is given by $x_2 = \frac{1}{N} p_2(1-p_1)$. All of these probabilities are summarized in table B5. Below we consider each within-patch pair identity in turn.

Transition probabilities

The transition probabilities, which are listed in table B6, are as follows:

1. *Transition probability,* $h_{13\leftarrow 3}$

The first transition probability, $h_{13\leftarrow 3}$, gives the probability that, of two randomly sampled individuals which descend from the same plasmid- and suppressor-carrying founder cell, one carries the plasmid and the other carries both traits after transmission. As both cells already carry both the suppressor and the plasmid, this probability is one, giving $h_{13\leftarrow 3} = 1$.

2. *Transition probability,* $h_{13\leftarrow 13}$

The transition probability, $h_{13\leftarrow 13}$, gives the probability that, of two randomly sampled individuals which descend from different founder cells, one carrying both traits and the other carrying the plasmid (regardless of whether it carries the suppressor or not), one carries the plasmid and the other carries both traits after transmission. As this is already the case, this probability is one, giving $h_{13\leftarrow 13} = 1$.

3. *Transition probability,* $h_{13\leftarrow 03}$

The transition probability, $h_{13\leftarrow 03}$, gives the probability that, of two randomly sampled individuals which descend from different founder cells, one carrying both traits and the other being plasmid-free, one carries the plasmid and the other carries both traits after transmission. The plasmid free individual can be infected with the plasmid upon contact with a plasmid carrier with probability β. This donor may be from the same strain as the sampled plasmid (and suppressor) carrying strain with probability $1/N$, or it may descend from a

separate plasmid-carrying strain with probability $\frac{N-2}{N} p_1$. This gives an overall conditional probability for transmission of $h_{13\leftarrow 03} = \beta\left(\frac{1}{N} + \frac{N-2}{N} p_1\right)$.

4. *Transition probability,* $h_{13\leftarrow 12}$

The transition probability, $h_{13\leftarrow 12}$, gives the probability that, of two randomly sampled individuals which descend from different founder cells, one carrying the plasmid (regardless of whether it carries the suppressor gene or not) and the other carrying the suppressor gene (and not the plasmid), one carries the plasmid and the other carries both traits after transmission. As before the plasmid free cell (which carries the suppressor) may be infected with the plasmid upon contact with a plasmid carrier with probability β. This donor may be from the same strain as the sampled plasmid-carrying strain with probability $1/N$, or it may descend from a separate plasmid-carrying strain with probability $\frac{N-2}{N} p_1$. This gives an overall conditional probability for transmission of $h_{13\leftarrow 12} = \beta\left(\frac{1}{N} + \frac{N-2}{N} p_1\right)$.

5. *Transition probability,* $h_{13\leftarrow 02}$

The transition probability, $h_{13\leftarrow 02}$, gives the probability that, of two randomly sampled individuals which descend from different founder cells, one plasmid-free (regardless of whether it carries the suppressor gene or not) and the other carrying the suppressor gene (and not the plasmid), one carries the plasmid and the other carries both traits after transmission. Both may be infected at the transmission stage upon contact with plasmid-carrying individuals at rate β^2. All such plasmid-carrying strains are different from the ancestral strains of the sampled individuals (neither of which carry the plasmid) by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which come

from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-2}{N}p_1\right)$.

Alternatively the sampled individuals may be infected by plasmid-carrying cells from two separate plasmid-carrying strains, this occurs with probability $\left(\frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right)$. From these the overall conditional probability is given by $h_{13\leftarrow 02}=\beta^2\left(\frac{1}{N}\frac{N-2}{N}p_1+\frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right)$.

6. *Transition probability,* $h_{13\leftarrow 2}$

The transition probability, $h_{13\leftarrow 2}$, gives the probability that, of two randomly sampled individuals which descend the same suppressor carrying, plasmid-free founder cell, one carries the plasmid and the other carries both traits after transmission. Both may be infected at the transmission stage upon contact with plasmid-carrying individuals at rate β^2. As above, all such plasmid-carrying strains are different from the ancestral strain of the sampled individuals (neither of which carry the plasmid) by virtue of their plasmid carriage. The sampled individuals may both be infected by plasmid-carrying cells which come from the same plasmid-carrying strain. This occurs with probability $\left(\frac{1}{N}\frac{N-1}{N}p_1\right)$. Alternatively the sampled individuals may be infected by plasmid-carrying cells from two separate plasmid-carrying strains, this occurs with probability $\left(\frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right)$. From these the overall conditional probability is given by $h_{13\leftarrow 2}=\beta^2\left(\frac{1}{N}\frac{N-1}{N}p_1+\frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right)$.

Pair probabilities for $\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{3j}{}^{\mathrm{t}}]$

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables B5 and B6) using equation (B5). This then gives the following equation for the pair probabilities:

$$\begin{aligned}\mathrm{E}[p_{1j}{}^{\mathrm{t}} p_{3j}{}^{\mathrm{t}}] = {} & \frac{1}{N} p_1 p_2 \\ & + \frac{N-1}{N}(p_1 p_2)(p_1) \\ & + \frac{N-1}{N}(p_1 p_2)(1-p_1)\beta\left(\frac{1}{N} + \frac{N-2}{N} p_1\right) \\ & + \frac{N-1}{N}(p_1)\big(p_2(1-p_1)\big)\beta\left(\frac{1}{N} + \frac{N-2}{N} p_1\right) \\ & + \frac{N-1}{N}(1-p_1)\big(p_2(1-p_1)\big)\beta^2\left(\frac{1}{N}\frac{N-2}{N} p_1 + \frac{N-2}{N}\frac{N-3}{N} p_1{}^2\right) \\ & + \frac{1}{N} p_2(1-p_1)\beta^2\left(\frac{1}{N}\frac{N-1}{N} p_1 + \frac{N-1}{N}\frac{N-2}{N} p_1{}^2\right).\end{aligned} \qquad [B6]$$

Calculation of $\mathrm{E}[p_{2j}{}^{t} p_{3j}{}^{t}]$

$\mathrm{E}[p_{2j}{}^{t} p_{3j}{}^{t}]$ is the probability that one samples an suppressor carrying individual (regardless of whether or not it also carriers the plasmid) and an individual who carries both the plasmid and the suppressor. We expand this probability in terms of conditional probabilities as:

$$\mathrm{E}[p_{2j}{}^{\mathrm{t}} p_{3j}{}^{\mathrm{t}}] = \sum_{i \in s}\left(f_{23\leftarrow i} x_i\right), \qquad (B7)$$

where $f_{23\leftarrow i}$ is the probability that two individuals, one carrying the plasmid and the other carrying both the plasmid and the suppressor, randomly sampled after transmission from a random patch of the population (a focal patch) descend from state of origin *i* before transmission (state of the cell before transmission), x_i is the probability of occurrence of the state of origin *i* and $S = \{3,23,22,2\}$. The index *i* represents the original state of the two cells, randomly sampled from the population, before transmission: *i*=3 refers to the case where both have the suppressor gene and the plasmid (and share the same founder cell), *i*=23 refers to the case where one has the suppressor and the other has both the plasmid and the suppressor gene (and the cells come from different founder cells), *i*=22 refers to the case where the cells descend from different founder cells, with one having the suppressor (regardless of whether or not it carries the plasmid as well) and the other having only the suppressor (i.e. it is plasmid-free) and finally *i*=2 refers to the case where both carry the suppressor gene but not the plasmid (and share the same founder cell). We now evaluate explicitly the probabilities going into equation (B7).

State of origin probabilities

The probability of occurrence of the state of origin *i* of the two descendant individuals is given by $x_{i,}$ where $i \in S$. The probability that the two individuals sampled both descend from the same plasmid- and suppressor-carrying founder strain is given by $x_3 = \frac{1}{N} p_1 p_2$ .The probability that the two individuals sampled descend from two separate founders strains where one has the suppressor gene (and not necessarily the plasmid) and the other has both the plasmid and the suppressor gene is given by $x_{23} = \frac{N-1}{N}(p_1 p_2)(p_2)$ .The probability that

the two individuals sampled descend from different founder cells, where one carries the suppressor (regardless of whether it carries the plasmid also) and the other carries only the suppressor gene is given by $x_{22} = \frac{N-1}{N}(p_2)(p_2(1-p_1))$. The probability that the two individuals sampled descend from the same founder strain, which carries only the suppressor gene, is given by $x_2 = \frac{1}{N} p_2(1-p_1)$. All of these probabilities are summarized in table B7. Below we consider each within-patch pair identity in turn.

Transition probabilities

The transition probabilities, which are listed in table B8, are as follows:

1. *Transition probability,* $f_{23\leftarrow 3}$

The first transition probability, $f_{23\leftarrow 3}$, gives the probability that, of two randomly sampled individuals which descend from the same plasmid- and suppressor-carrying founder cell, one carries the suppressor and the other carries both traits after transmission. As both cells already carry both the suppressor and the plasmid, this probability is one, giving $f_{23\leftarrow 3} = 1$.

2. *Transition probability,* $f_{23\leftarrow 23}$

The transition probability, $f_{23\leftarrow 23}$, gives the probability that, of two randomly sampled individuals which descend from different founder cells, one carrying both traits and the other carrying the suppressor gene (regardless of whether it also carries the plasmid or not), one carries the suppressor and the other carries both traits after transmission. As this is already the case, this probability is one, giving $f_{23\leftarrow 23} = 1$.

3. *Transition probability,* $f_{23\leftarrow 22}$

The transition probability, $f_{23\leftarrow 22}$, gives the probability that, of two randomly sampled individuals which descend from different founder cells, one carrying the suppressor gene (regardless of whether it carries the plasmid also) and the other only the suppressor gene, one carries the suppressor and the other carries both traits after transmission. The plasmid free cell may be infected with the plasmid upon contact with a plasmid carrier with probability β. This donor will come from a separate strain to the sampled individuals $\frac{N-2}{N} p_1$. This gives an overall conditional probability for transmission of $f_{23\leftarrow 22} = \beta\left(\frac{N-2}{N} p_1\right)$.

4. *Transition probability,* $f_{12\leftarrow 2}$

The transition probability, $f_{12\leftarrow 2}$, gives the probability that, of two randomly sampled individuals which descend from the same plasmid-carrying, suppressor-free founder strain, one carries the plasmid and the other carries both traits after transmission. One of the cells may be infected with the plasmid upon contact with a plasmid carrier with probability β. This donor will necessarily come from a different strain to the sampled strain $\frac{N-1}{N} p_1$. This gives an overall conditional probability for transmission of $f_{23\leftarrow 2} = \beta\left(\frac{N-1}{N} p_1\right)$.

Pair probabilities for $\mathrm{E}[{p_{2j}}^t {p_{3j}}^t]$

As mentioned above, we can calculate the overall probabilities by combining the conditional probabilities given above (and in tables B7 and B8) using equation (B7). This then gives the following equation for the pair probabilities:

$$
\begin{aligned}
\mathrm{E}[{p_{2j}}^{t}{p_{3j}}^{t}] = {} & \frac{1}{N}p_1p_2 \\
& + \frac{N-1}{N}(p_1p_2)(p_2) \\
& + \frac{N-1}{N}(p_2)\big(p_2(1-p_1)\big)\beta\left(\frac{N-2}{N}p_1\right) \\
& + \frac{1}{N}p_2(1-p_1)\beta\left(\frac{N-1}{N}p_1\right).
\end{aligned}
\tag{B8}
$$

Chapter 5 Appendix B Supplementary Tables

Table B1. The calculation of x_i for $\mathrm{E}[{p_{1j}}^{t}{p_{1j}}^{t}]$.

x_i	Probability
x_1	$\frac{1}{N}p_1$
x_{11}	$\frac{N-1}{N}{p_1}^2$
$x0$	$\frac{1}{N}(1-p_1)$
x_{00}	$\frac{N-1}{N}(1-p_1)^2$
x_{10}	$2\frac{N-1}{N}p_1(1-p_1)$

Table B2. The calculation of $t_{11\leftarrow i}$ for $\mathrm{E}[{p_{1j}}^{\mathrm{t}}{p_{1j}}^{\mathrm{t}}]$.

$t_{11\leftarrow i}$	Transition probability
$t_{11\leftarrow 1}$	1
$t_{11\leftarrow 11}$	1
$t_{11\leftarrow 0}$	$\beta^2\left(\frac{1}{N}\frac{N-1}{N}p_1+\frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right)$
$t_{11\leftarrow 00}$	$\beta^2\left(\frac{1}{N}\frac{N-2}{N}p_1+\frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right)$
$t_{11\leftarrow 10}$	$\beta\left(\frac{1}{N}+\frac{N-2}{N}p_1\right)$

Table B3. The calculation of x_i for $\mathrm{E}[{p_{1j}}^{\mathrm{t}}{p_{2j}}^{\mathrm{t}}]$.

x_i	Probability
x_2	$\frac{1}{N}p_2(1-p_1)$
x_{22}	$\frac{N-1}{N}\left(p_2(1-p_1)\right)^2$
x_3	$\frac{1}{N}p_1p_2$
x_{33}	$\frac{N-1}{N}(p_1p_2)^2$

Table B4. The calculation of $g_{12\leftarrow i}$ for $\mathrm{E}[{p_{1j}}^{\mathrm{t}} {p_{2j}}^{\mathrm{t}}]$.

$g_{12\leftarrow i}$	Transition probability
$g_{12\leftarrow 2}$	$\beta\left(\frac{N-1}{N} p_1\right)$
$g_{12\leftarrow 22}$	$\beta\left(\frac{N-2}{N} p_1\right)$
$g_{12\leftarrow 3}$	1
$g_{12\leftarrow 33}$	1

Table B5. The calculation of x_i for $\mathrm{E}[{p_{1j}}^{\mathrm{t}} {p_{3j}}^{\mathrm{t}}]$.

x_i	Probability
x_3	$\frac{1}{N} p_1 p_2$
x_{13}	$\frac{N-1}{N}(p_1 p_2)(p_1)$
x_{03}	$\frac{N-1}{N}(p_1 p_2)(1-p_1)$
x_{12}	$\frac{N-1}{N}(p_1)\left(p_2(1-p_1)\right)$
x_{02}	$\frac{N-1}{N}(1-p_1)\left(p_2(1-p_1)\right)$
x_2	$\frac{1}{N} p_2(1-p_1)$

Table B6. The calculation of $h_{13\leftarrow i}$ for $\mathrm{E}[{p_{1j}}^{t}{p_{3j}}^{t}]$.

$h_{13\leftarrow i}$	Transition probability
$h_{13\leftarrow 3}$	1
$h_{13\leftarrow 13}$	1
$h_{13\leftarrow 03}$	$\beta\left(\frac{1}{N}+\frac{N-2}{N}p_1\right)$
$h_{13\leftarrow 12}$	$\beta\left(\frac{1}{N}+\frac{N-2}{N}p_1\right)$
$h_{13\leftarrow 02}$	$\beta^2\left(\frac{1}{N}\frac{N-2}{N}p_1+\frac{N-2}{N}\frac{N-3}{N}{p_1}^2\right)$
$h_{13\leftarrow 2}$	$\beta^2\left(\frac{1}{N}\frac{N-1}{N}p_1+\frac{N-1}{N}\frac{N-2}{N}{p_1}^2\right)$

Table B7. The calculation of x_i for $\mathrm{E}[{p_{2j}}^{t}{p_{3j}}^{t}]$.

x_i	Probability
x_3	$\frac{1}{N}p_1p_2$
x_{23}	$\frac{N-1}{N}(p_1p_2)(p_2)$
x_{22}	$\frac{N-1}{N}(p_2)\left(p_2(1-p_1)\right)$
x_2	$\frac{1}{N}p_2(1-p_1)$

Table B8. The calculation of $f_{23\leftarrow i}$ for $\mathrm{E}[{p_{2j}}^{t}\,{p_{3j}}^{t}]$.

$f_{23\leftarrow i}$	Transition probability
$f_{23\leftarrow 3}$	1
$f_{23\leftarrow 23}$	1
$f_{23\leftarrow 22}$	$\beta\left(\frac{N-2}{N}p_1\right)$
$f_{23\leftarrow 2}$	$\beta\left(\frac{N-1}{N}p_1\right)$

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10. Curriculum Vitae

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2009-2012	**University of Zürich**
	Institute of Evolutionary Biology and Environmental Studies
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	Thesis title: The role of Horizontal Gene Transfer in Microbial Social Evolution.
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2008 - 2009	**University of Oxford**
	M.Sc. Biology (Integrative Bio-science) (Distinction)
	M.Sc. Thesis 1: The consequences of canalisation in Collembola.
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2004 – 2008	University of Dublin, Trinity College
	B.A. Natural Science mod. Zoology (First class hons)
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Publications

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